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PRIMARY TRISOMICS OF *TRITICUM MONOCOCCUM*

THEIR PRODUCTION, IDENTIFICATION, CYTOLOGICAL BEHAVIOUR, PHENOTYPES, BREEDING BEHAVIOUR AND FERTILITY

BY

NAM-SOO KIM

©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF GENETICS

EDMONTON, ALBERTA
FALL, 1990

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We the co-authors of the paper " Genetic and cytogenetic analyses of the A genome of *Triticum monococcum*. III. Cytology, breeding behaviour, fertility, and morphology of autotriploids. (Can. J. Genet. Cytol. Vol 28. 1986. pp 867 - 887)" permit Mr. Nam-Soo Kim to use Fig. 4. (A) Pairing and crossing-over associated in trivalents at pachytene and the resulting configurations at diakinesis. (B) Oberserved frequencies of the coorientations of trivalents at metaphrase I and their subsequent anaphase I segregations." in his thesis. The figure is numbered Fig. 2. in the thesis.

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Primary trisomics of *Triticum monococcum:* their production, identification, cytological behaviour, phenotypes, breeding behaviour and fertility submitted by Nam-Soo Kim in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Date: June 1, 1990

DEDICATION

" The history of the earth is recorded in the layers of its crust; the history of all organisms is inscribed in the chromosomes"

Hitoshi Kihara

This thesis is dedicated to my father and late mother, my brothers and sister, and my wife Hea-Sook

ABSTRACT

Cytogenetic studies in *Triticum monococcum* ($2n = 2x = 14$; *AA*) were initiated by generating a series of primary as well as double and triple trisomics. These aneuploids were generated from autotriploids derived from crosses between induced autotetraploids and a diploid progenitor.

The chromosomes of *T. monococcum* were morphologically very similar and therefore could not be unequivocally identified using standard techniques. Ag-NOR staining and *in situ* hybridization, using a rDNA probe, revealed that two of the seven chromosome pairs in *T. monococcum* carry NORs in the terminal regions of their short arms. The labelling pattern in the SAT chromosome pair that hybridized lightly is, in all likelihood, 1A; the pair that hybridized heavily is likely the chromosome pair 5A. Genes coding for 5S rRNA were found to be located on the NOR-carrying chromosomes 1A and 5A, and in juxtaposition to the NORs.

A comparison of the C-banding patterns of the seven chromosomes of the *T. monococcum* genome with those of the A genome in *T. aestivum* permitted identification of chromosomes 1A, 2A, 3A, 5A, and 7A. The two others (4A and 6A) possessed C-banding patterns that were not equivalent to those of any of the chromosomes in the A genome of the polyploid wheats and therefore were identified by other means.

Cytological analysis permitted the identification of six of the seven possible primary trisomics. Trisomics for chromosome 3A were not found among the trisomic lines analyzed cytologically.

Analysis of meiotic behaviour revealed that, with the exception of trisomics for chromosome 7A, the chromosome present in triple dose in all other trisomics formed either a bivalent plus a univalent or a trivalent (always V-shaped) at diakinesis - metaphase I in approximately equal proportions. Trisomics for chromosome 7A formed a bivalent plus a univalent or a trivalent in approximately a

1:2 ratio. About 99% of the anaphase I segregations in all the trisomics were 7 - 8, suggesting that primary trisomics in *T. monococcum* form n and $n+1$ meiotic products in equal proportions.

All the trisomics differed phenotypically from their diploid progenitors. Single primary trisomics for chromosomes 3A and 7A produced distinct morphological features on the basis of which they could be distinguished. Trisomics for chromosome7A were identified on the basis of their distinct phenotype, viz., the small narrow heads and small narrow leaves. The phenotypes of the double and triple trisomics deviated to a greater extent than those of the single trisomics.

Less than 50% of the progeny of all primary trisomics were trisomics themselves. Trisomic progeny were not produced in diploid♀ x trisomic♂ crosses, indicating that functional $n+1$ male gametes were not generated. Diploid as well as trisomic progeny were produced in the reciprocal crosses, and upon self-fertilization of the trisomics. The average frequency of trisomic progeny was 9.9%.

The fertility of primary trisomics ranged from 3.8% in trisomics for chromosome 1A to 40.6% in trisomics for chromosome 2A, and was significantly less than that of diploids (99.6%). The breeding behaviour and low fertility of these trisomics make their maintenance and use in cytogenetic analyses difficult.

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I would like to express my sincerest gratitude to Dr. J. Kuspira without whose tutelage and direction I would not have been able to fulfill my objectives. The assistance of Dr. R. Bhambhani and Mr. J. Maclagan in overcoming my difficulties with the language as well as their friendship and encouragement throughout my stay in Edmonton are also greatly appreciated.

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INTRODUCTION

Bread wheat, *Triticum aestivum* L., and macaroni wheat, *Triticum turgidum* L., two of the world's most economically important crops, have been cultivated for at least 8,000 and 10,000 years, respectively (Helbaek 1959; Morris and Sears 1967). The phylogenies as well as the genetics and cytogenetics of these wheats have been the focus of much attention and speculation since the first decade of this century. Initial cytological studies by Sakamura (1918) and Kihara (1919, 1924) established that some of the wheat species, of which *Triticum monococcum* L. is a representative, were diploids with 14 chromosomes. Some others, of which *T. turgidum* is a representative, were allotetraploids with 28 chromosomes and still others, like *T. aestivum*, were allohexaploids with 42 chromosomes. Kihara (1924) showed that in addition to other genomes, the genome of *T. monococcum*, which he designated as A, was present in the representative species of both polyploids. Based on cytogenetic studies Kihara (1924) gave the genomic formula AA to *T. monococcum*, AABB to *T. turgidum* and AABBDD to *T. aestivum*. These results implicated *T. monococcum* as the source of the A genome in polyploid wheats. McFadden and Sears (1944, 1946) established that *Aegilops squarrosa* (=*Triticum tauschii*) is the source of the D genome. The source of the B genome is still currently unknown (Kerby and Kuspira 1987).

Genetic analysis in the wheats, as in other eukaryotes, requires the availability of true-breeding lines with different traits of the characters that are being studied. Cytogenetic studies in these species require either mutants with numerical or structural rearrangements of chromosomes, euploids such as chromosome substitution lines or chromosome addition lines.

The hexaploid wheat species *T. aestivum* ($2n=6x=42$) has been studied very extensively both cytologically and genetically using nullisomics, monosomics, chromosome substitution lines and nullisomic-tetrasomic lines (Sears 1948, 1969; Morris and Sears 1967; Hart 1982, 1988; McIntosh 1988a, 1988b; Sharp *et al.* 1989). Such studies have also been carried out in the tetraploid wheat species *T. turgidum* ($2n=4x=28$) albeit less extensively because aneuploids were not available until a few years ago (Suseelan *et al.* 1982; Joppa *et al.* 1983,1987; du Cros *et al.* 1983; Johnson *et al.* 1983; Harberd *et al.* 1985; Joppa and Williams 1988; Sridevi and Goud 1988; Obanni *et al.* 1989). However, similar studies in the diploid wheat species including *T. monococcum*($2n=2x=14$), have been limited (Sears 1948; McIntosh 1988a). For example, cytogenetic studies in this diploid species have been limited to two brief analyses by Smith (1936), and Moseman and Smith (1954) using a line with an unidentified telocentric chromosome, which is no longer available. Our knowledge of the genetics of *T. monococcum* is also meagre (Smith 1939, 1947; Sharma and Waines 1980; Kuspira *et al.* 1986a, 1989), with most of the information having accrued in the last decade .

We embarked on a program that would enable us to cytogenetically and genetically characterize the genome of *T. monococcum*. Since the requisite mutants for cytogenetic analyses were not available we had to establish the necessary aneuploids and chose specifically to generate primary trisomics and telotrisomics for several reasons. Mutants with chromosomal rearrangements have rarely been used in plant species for cytogenetic purposes, primarily because they are more difficult to identify than aneuploids. With few exceptions, the only aneuploids that can be produced and maintained in diploid plant species are trisomics. In most such species, a complete series of primary trisomics can be established, from which it is possible to produce secondary trisomics and telotrisomics for each arm of each chromosome. Moreover, one can isolate trisomics in which the extra chromosome is a telocentric with only a portion of one arm present. These trisomics can be identified using standard cytological and current molecular procedures. Once identified, they can be used to carry out more precise genetic and cytogenetic studies.

One of the objectives of the investigations reported on in this thesis was to produce a complete series of primary trisomics in *T. monococcum*. As a first step in the generation of these primary trisomics, autotetraploids were induced in one of the strains of this diploid species. The polyploids were subsequently used to establish autotriploids, a necessary intermediate in the production of the desired aneuploids. These autotriploids were then utilized in a conventional manner to establish a complete series of primary trisomics and other types of aneuploids. A second objective was to identify the chromosomes in the A genome and the chromosome present in triplicate in trisomics using conventional and molecular procedures. Each chromosome in the diploid complement and in each trisomic was identified on the basis of the C-banding patterns of the chromosomes. Ag-NOR staining and *in situ* hybridization using rDNA probes were also used to substantiate the identification of the chromosomes and trisomics based on C-banding analysis. A third objective was to characterize these trisomics and related aneuploids on the basis of their general morphology, meiotic chromosome behaviour, fertility and breeding behaviour.

LITERATURE REVIEW

I. Phylogeny of the polyploid wheats.

The genus *Triticum* is one of 14 genera that are included in the *Triticeae* tribe. A comparative analysis of plant morphologies allowed Schulz (1913) to place each species of *Triticum* into one of three groups: Einkorn, Emmer, or Dinkel, represented by *T. monococcum*, *T. turgidum*, and *T. aestivum*, respectively. Sakamura (1918) established the somatic chromosome number of each of the three groups to be 14, 28, and 42 chromosomes, respectively. This led Kihara (1919) to postulate that the different species comprising the genus form a series with three levels of ploidy: Einkorns being diploids ($2n=2x=14$), Emmers tetraploids ($2n=4x=28$), and the Dinkels hexaploids ($2n=6x=42$). Moreover, these and subsequent studies by Sax and Sax (1924), Kihara (1944), McFadden and Sears (1944, 1946), and others showed that each of the genomes in *Triticum*, as well as in the closely related genus *Aegilops*, possessed seven chromosomes. Analysis of the meiotic chromosome behaviour in *T. turgidum* x *T. monococcum* and *T. aestivum* x *T. turgidum* hybrids indicated that the tetraploid and hexaploid wheats were allopolyploids, allowing Kihara (1919, 1924) to ascribe the genomic formulae AA, AABB, and AABBDD to *T. monococcum*, *T. turgidum*, and *T. aestivum*, respectively. These and other cytological studies (Melburn and Thompson 1927; Kihara and Nishiyama 1928) indicated that the A genome in the polyploid wheats was derived from an Einkorn wheat, specifically *T. monococcum*. C-banding studies by Gill and Kimber (1974a) and purothionin analyses by Fernandez de Caleya *et al.* (1976), Jones *et al.* (1982), and Kerby and Kuspira (1988) have confirmed this conclusion. However, a variety of different studies by Chapman *et al.* (1976), Dvořák (1976), Konarev (1983), Nath *et al.* (1983), and Kerby and Kuspira (1988) have shown that *Triticum urartu* is very closely related to *T. monococcum* and, in all likelihood, possesses the A genome. If *T. monococcum* and *T. urartu* are indeed different species, *T. monococcum* can no longer be considered to be the unequivocal source of the A genome.

Based upon a comparison of external morphological features and chromosome morphologies, Pathak (1940) proposed that *Ae. squarrosa* is the donor of the D genome in *T. aestivum*. Cytological, morphological, and fertility studies of allohexaploids derived from the cross of *T. turgidum* x *Ae. squarrosa* (McFadden and Sears 1946), C-banding studies (Gill and Kimber 1974a) and purothionin analyses (Fernandez de Caleya *et al.* 1976; Jones *et al.* 1982) of *Ae. squarrosa* have all verified Pathak's proposal.

The source of the B genome in the polyploid wheats has been and remains equivocal and controversial. Studies by Riley *et al.* (1958), Suemoto (1968), Kimber and Athwal (1972), Johnson (1972), Feldman (1978), Kushnir and Halloran (1981), Nath *et al.* (1983), Kerby (1986) and others implicated one or another of six species (*Aegilops bicornis, Aegilops longissima, Aegilops searsii, Aegilops sharonensis, Aegilops speltoides*, and *T. urartu*) as the likely donor of the B genome to the polyploid wheats. The findings of Dvořák (1976), Chapman *et al.* (1976), and Kerby and Kuspira (1988) clearly indicate that *T. urartu* should be excluded from the list of possible B genome donors. Thus indicating that in all likelihood the B genome is derived from one or more of the five species in the *Sitopsis* section of *Aegilops*. The results obtained by Feldman (1978), Nath *et al* (1983, 1984) Jones *et al.* (1982), and Kerby (1986) strongly suggest that if the B genome is monophyletic in origin, *Ae. searsii* is the most likely source of this set of chromosomes. The possibility that the B genome in the polyploid wheats could have a polyphyletic origin should not be ruled out.

A widely and currently accepted phylogeny of the polyploid wheats is given in Fig. 1.

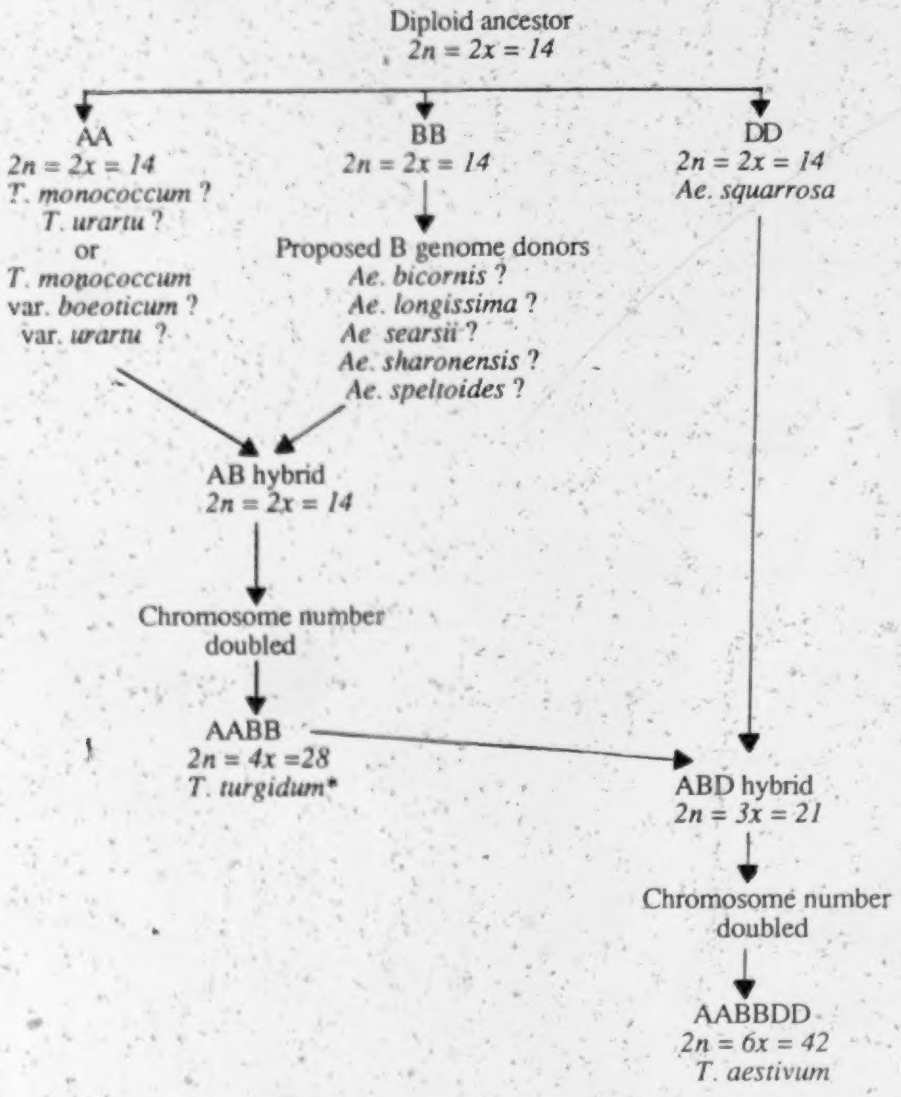

Diploid ancestor
2n = 2x = 14
AA
2n = 2x = 14
T. monococcum ?
T. urartu ?
or
T. monococcum
var. boeoticum ?
var. urartu ?
BB
2n = 2x = 14
Proposed B genome donors
Ae. bicornis ?
Ae. longissima ?
Ae searsii ?
Ae. sharonensis ?
Ae. speltoides ?
DD
2n = 2x = 14
Ae. squarrosa
AB hybrid
2n = 2x = 14
Chromosome number doubled
AABB
2n = 4x =28
T. turgidum*
ABD hybrid
2n = 3x = 21
Chromosome number doubled
AABBDD
2n = 6x = 42
T. aestivum

Fig. 1. Probable origin of *T. turgidum* and *T. aestivum*. Each capital letter represents a genome composed of seven chromosomes.
* In all likelihood, a cultivated form of *T. turgidum* hybridized with *Ae. squarrosa* to form the ABD hybrid. (Modified from Kerby *et al.* 1988).

II. *Triticum monococcum*

A. Taxonomical status and geographical distribution

Triticum monococcum ($2n=2x=14$, AA) consists of three varieties: *monococcum*, *boeoticum*, and *sinskajae* (Kimber and Feldman 1987). It is not clear whether *T. urartu*. L., which is believed to be closely related to *T. monococcum*, is another variety of *T. monococcum* or a different species altogether (Kerby and Kuspira 1987).

The var. *monococcum* was domesticated by neolithic man and is still cultivated in some very small regions in Turkey, Greece, Yugoslavia, and Italy that have poor soil (Kimber and Feldman 1987). Although the var. *boeoticum* and *T. urartu* are more or less genetically isolated from each other, they are recognized as the wild forms of diploid wheat because both carry two notable characters that distinguish them from domesticated wheats: (1) disarticulated spikelets and (2) kernels that are tightly enclosed by the palea, lemma and glumes (Sharma and Waines 1980; Waines 1983). Both these features make the wild wheats difficult to harvest and thresh. The var. *sinskajae* differs from the domesticated varieties of *monococcum* as well as the two wild forms in that it has soft glumes which makes it easy to thresh. The two wild diploid forms together with the var. *sinskajae* are distributed in the N.E. Mediterranean and W. Asiatic regions of poor soil at an altitude of 100 - 1600 m (Kimber and Feldman 1987).

B. Cytology

Various studies since 1939 have shown that *T. monococcum* exhibits intraspecifc variation with respect to the morphologies of its chromosomes. Pathak (1940), Camara (1943), and Giorgi and Bozzini (1969b) reported that two of the seven chromosome pairs in this species possessed small satellites. Riley *et al.* (1958), Upadhya and Swaminathan (1963), and Coucoli and Skorda (1966) observed only one pair of chromosomes with minute satellites. Waines and Kimber (1973) reported that both variation in satellite size and the number of chromosome pairs with satellites depended on the geographical origin of the lines studied. Studies by Coucoli and Skorda (1966) and Giorgi and Bozzini (1969b) showed that the seven chromosomes in the genome varied in length from 8.36 to 12.29*u*m and 6.28 to 8.32 *u*m, respectively. Studies by Sears (1958), Gill *et al.* (1963) and others reported other size variations. The differences in chromosome sizes reported in the different studies may be attributed to the use of different techniques and staining procedures and measurements of chromosome length at different substages of mitosis and meiosis. Giorgi and Bozzini (1969b) observed 1 ST (subterminal), 4 SM (submedian), and 2 M (median) chromosome pairs in the diploid complement of *T. monococcum* with one of the SAT (satellited) pairs being ST and the other being SM. The observations of Kerby and Kuspira (1988) were in agreement with those of Giorgi and Bozzini (1969b). The consensus of these studies is that the chromosomes in the diploid complement of this species are very similar in gross morphology.

Gill and Kimber (1974a) showed that the C-banding patterns of the chromosomes in the *T. monococcum* genome are similar to those of the A genome in *T. aestivum*. Although the C-bands were small, they were located in the telomeric as well as the centromeric regions of the chromosomes. Shang *et al.* (1989), using an HCl-KOH-Giemsa banding technique, reported similar banding patterns for the chromosomes of *T. monococcum*. They also showed that the distribution of heterochromatin along the chromosomes of the A genome was different in the closely related wild and cultivated diploid and polyploid wheats. Moreover, they reported that this variation in heterochromatin distribution was related to geographic distribution and to natural and artificial selection.

Meiotic chromosome pairing in *T. monococcum* is highly regular. Kihara (1919) and others observed seven bivalents in the meiocytes. Asynaptic or desynaptic mutants have not been reported as yet (Kuspira *et al.* 1985). In induced autotetraploids in this species, Kuspira *et al.* (1985) observed an average of 0.62 univalents, 9.86 bivalents, 0.23 trivalents, and 1.74 quadrivalents per meiocyte at metaphase I (MI). A 14-14 distribution of chromosomes at anaphase I (AI) was observed in 67% of the meiocytes of these polyploids. The remaining meiocytes showed all the other possible distributions. In autotriploids of this Einkorn species, an average of 2.65 univalents, 2.60 bivalents, and 4.3 trivalents per meiocyte at MI were observed (Kuspira *et al.* 1986b). Fifty-two percent of the meiocytes in these polyploids revealed a 10-11 distribution of chromosomes at AI while the remainder showed all other possible distributions. Gillies *et al.* (1987), using electron microscopy of synaptonemal complex spreads, observed a mean of 3.59 multivalents per meiocyte at zygotene-pachytene.

C. Genetics

Few investigations have been conducted on the modes of inheritance of characters in *T. monococcum*. As a consequence, our understanding of the genetic heritage of this species is meagre. Smith (1939) demonstrated that each of several chlorophyll mutants, which no longer exist, were determined by recessive mutations. He did not determine whether these recessives were allelic or not. Sharma and Waines (1980) showed that tough vs. fragile rachis is determined by the interaction of two independently inherited allele pairs with the allele for fragility dominant to that for toughness at each locus. In 1983, Waines showed that glume hardness is determined by a single gene with the allele for soft being recessive to that for hard. Kuspira *et al.* (1986a) demonstrated that spring vs. winter growth habit is determined by a single multiple allelic series which is probably at the Vrn 1 locus on chromosome 5A. In 1989, Kuspira *et al.* reported that 12 characters (awn length, awn on outer glume, false glume, glume colour, glume hardness, glume pubescence, growth habit, head type, kernel colour, leaf pubescence, node colour, and node pubescence) were each determined by a single major gene. A multiple allelic series was shown to exist at each of the glume pubescence(Hg) and node pubescence(Hn) loci. The genes Bg (glume colour) and Hg were shown to be the same short distance (6.1 map units) apart as in *T. aestivum*. The genes for glume hardness(Sg), lemma awn length(La), false glume(Fg), and head type(Lh) are also very closely linked, with the outside markers being only four map units apart.

The three genes, Sr21, Sr22, and Sr35 for resistance to stem rust *Puccinia graminis tritici*, have been transferred from *T. monococcum* to *T. aestivum* and mapped to chromosome 2A (The 1973), 7A (Kerber and Dyck 1973), and 3A (McIntosh *et al.* 1984), respectively, using monosomic analysis in Chinese spring.

Sears (1948) noted that natural phenotypic and genetic variability in *T. monococcum* are limited. The investigations by McIntyre (1988) at 16 isozymic loci in four accessions of different geographical origin and Smith-Huerta *et al.* (1989) at the same number of such loci in 17 accessions from different geographic regions confirmed this long standing observation. They showed that the mean number of alleles per locus in this species is 1.1 and 1.2, respectively. The observation by Kuspira *et al.* (1989) on morphological characters in 460 true-breeding lines also support the initial conclusion.

III. Trisomics

A. History, trisomic types, and terminology

Lutz (1909) first found several plants in *Oenothera lata* ($2n=2x=14$) and two plants among hybrids from the cross *O. lata* x *O. gigas* that contained an extra chromosome. These plants, which were referred to as trisomics by Blakeslee (1921a), are representative aneuploids, a term coined by Tackholm (1922) to describe organisms whose chromosome numbers were not a multiple of the basic number charateristic of the species to which they belonged.

In 1915 Avery discovered a mutant in *Datura stramonium* ($2n=2x=24$) that he called Globe. This mutant was characterized by many phenotypic differences from the diploids; it behaved as a dominant trait and did not breed true. The determiner of Globe was transmitted to the progeny almost solely via the female gametes, with the mutant phenotype occurring in an unexpected proportion (25%) of the progeny from Globe♀ x nomal♂ crosses. Only 2% of the progeny of reciprocal crosses were of the Globe phenotype. Eleven other mutants, each with different morphologies from Globe and one other but with breeding behaviours similar to that of Globe were discovered by 1919 (Blakeslee 1921b; Blakeslee and Avery 1919). Blakeslee *et al.* (1920) revealed that the 12 mutants each contained an extra chromosome in their somatic chromosome complements. Blakeslee (1921a) coined the term "primary trisomics" for these aneuploids in which the extra chromosome was a normal chromosome of the complement. Subsequent studies in *D. stramonium* showed that the extra chromosome in trisomics could be an isochromosome (Belling and Blakeslee 1924), a tertiary chromosome comprising two arms from non-homologous chromosomes (Belling and Blakeslee 1926) or a telocentric chromosome (Belling 1927); these aneuploids were referred to as secondary trisomics (Blakeslee 1924; Goodspeed and Avery 1939), tertiary trisomics (Belling and Blakeslee 1926; Rhodes 1933), and telotrisomics (Khush and Rick 1968b), respectively. In 1927 Blakeslee discovered an aneuploid plant that did not fit the description of any of these three groups. It lacked one complete chromosome but was compensated for by the presence of two tertiary chromosomes. One of these tertiary chromosomes had one of the arms of the missing chromosome while the second carried the other arm. Khush and Rick (1967a) designated such aneuploids as compensating trisomics. It should be noted that other terms, no longer in use and not discussed here, have also been coined for these different trisomics.

Subsequent to these classic studies in *Datura*, trisomics have been found or generated in many other diploid as well as in polyploid plant species including durum wheat (See Khush 1973; Simeone *et al.* 1983; Kuspira *et al.* 1986b).

Aneuploids, particularly trisomics, have been found and studied in only a few animal species. Bridges (1916) first discovered aneuploids in *Drosophila melanogaster*, specifically for sex chromosomes. Some of these were monosomics (AAXO) and others trisomics (AAXXY or AAXXX). The correlation of the sex phenotypes of these and normal individuals with their karyotypes allowed Bridges to determine the location of the sex determining genes in this species. By studying the transmission patterns of an X-linked pair of alleles for eye color in normal females and males as well as in monosomic (AAXO) and trisomic (AAXXY and AAXXX) flies, Bridges proved that chromosomes carry genes.

Aneuploids in *Homo sapiens* were not identified until 1959 when Lejeune *et al.* showed that individuals with Down's syndrome were aneuploids and trisomic for a small acrocentric chromosome (now known as chromosome 21). In the same year Jacobs *et al.* found the first trisomic female with three XXX chromosomes (AAXXX) whereas Jacobs and Strong showed that males with Klinefelter's syndrome were trisomic with the AAXXY chromosome constitution. In 1965 Jacobs *et al.* discovered trisomics with an AAXYY chromosome constitution.

Aside from trisomy for chromosome 21, only 5 other autosomal trisomics are compatible with life, for at least a short period. These include trisomics for chromosome 8 (Caspersson *et al.* 1972), 9 (Feingold and Atkins 1973), 13 (Patau *et al.* 1960), 18 (Edwards *et al.* 1960), and 22 (Punnett *et al.* 1973). The individuals that are trisomic for each of these chromosomes express specific syndromes. All other primary trisomics cause embryonic abortion (Hassold and Jacobs 1984). Subsequent to these discoveries numerous other aneuploid types have been identified and studied in man. As well in the mouse, *Mus musculus* ($2n=2x=40$), only a few of the possible trisomics, usually those for small and medium sized chromosomes are compatible with life. Of the 19 possible autosomal primary trisomics, only those individuals that are trisomic for chromosome 19(shortest autosome) survive until the early neonatal period. All others die before birth (White *et al.* 1974; Gropp *et al.* 1975; Epstein 1985). Mice that are trisomic for the sex chromosomes e.g. AAXXY, AAXYY, and AAXXX are compatible with life as in humans (Welshons and Russell 1959; Cattanach and Pollard 1969). Trisomics have also been found to occur sporadically in fowl (Bloom 1969), brook trout (Davisson *et al.* 1972), chimpanzees (McClure *et al.* 1969; Benirschke *et al.* 1974), cat (Thuline and Norby 1961), and water vole (Fredga 1968).

B. Sources of primary trisomics

1. Autotriploids

The most common and reliable sources of primary trisomics are autotriploids which arise spontaneously in a few plant species e.g. *Oryza sativa* (Ramanujam 1937; Watanabe *et al.* 1969; Khush *et al.* 1984) and *Lycopersicum esculentum* (Rick and Barton 1954). In *Zea mays* (McClintock 1929), *Hordeum spontaneum* (Tsuchiya 1960), *Secale cereale* (Kamanoi and Jenkins 1962), *Lotus pedunculatus* (Chen and Grant 1968a), *Lolium perenne* (Meijer and Ahloowalia 1981) and most other plant species they are derived from crosses between autotetraploids and diploids. Autotriploids usually produce a complete array of meiotic products from n through all the possible unbalanced types ($n+1$, $n+2$, etc) to $2n$ (Belling and Blakeslee 1922; Lesley 1926; Goodspeed and Avery 1939; Kuspira *et al.* 1986b; and others). Moreover the different $n+1$ gametes, each containing an extra copy of a different (non-homologous) chromosome of the genome, are present in approximately equal proportions. As a result, a complete series of primary trisomics have been generated from large progenies of autotriploids of *L. esculentum* (Lesley 1926; Khush *et al.* 1984), *Nicotiana sylvestris* (Goodspeed and Avery 1939), *H. spontaneum* (Tsuchiya 1960), *Avena strigosa* (Rajhathy 1975), *L. perenne* (Meijer and Ahloowalia 1981), *Beta vulgaris* (Romagosa *et al.* 1986) and many other plant species. However in ryegrass (Myers 1944), spinach (Janick *et al.* 1959), barley (Tsuchiya 1960), pearl millet (Gill *et al.* 1970), tomato (Rick 1971), as in most plant species, only a fraction of all the other possible aneuploid progeny types were realized. Only those trisomics with the least chromosomal and genic imbalance, e.g. primary single ($2n+1$), double ($2n+2$), and triple ($2n+3$) trisomics are compatible with life. For example in 1960 Tsuchiya found that among 126 progeny of autotriploid barley plants 29, 59, 22, and 6 were $2n$, $2n+1$, $2n+2$, and $2n+3$, respectively. The remainder included telotrisomics, $2n+1$+telocentric, autotriploids and unidentified types. A few species including *Petunia hybrida* (Rick 1971) and *Collinsia heterophylla* (Dhillon and Garber 1960) are highly tolerant of aneuploidy and as a result the complete range of viable aneuploids has been obtained in these species.

On rare occassions primary trisomics have been obtained from autotetraploids. Randolf and Fisher (1939) obtained one such plant from among 17,165 offspring of autotetraploid *Zea mays*. Hermsen (1969) recovered several trisomics from the progenies of colchicine-induced autotetraploids of *Solanum chacoense*.

2. Normal disomics

Primary trisomics, as well as other aneuploids, appear spontaneously at a low rate among the progenies of normal disomics. Belling and Blakeslee (1924) found that eight of 1137 meiocytes in diploid *Datura* showed 11-13 segregation at AI. Therefore about 0.4% of the meiotic products were expected to be *n+1* types. All 12 primary trisomics in *D. stramonium* ($2n=2x=24$) arose spontaneously in diploids (Blakeslee 1921a). They have also arisen spontaneously in *Antirrhinum majus* (Stubbe 1934), *S. cereale* (Takagi 1935), *L. esculentum* (Rick 1945), *Gossypium hirsutum* (Kohel 1966), *Z. mays* (Ghidoni *et al.* 1982), and *Plantago lagopus* (Sharma *et al.* 1985). Nondisjunction in either the germ line cells or during meiosis is the most likely means by which the requisite *n+1* gametes can be generated in diploids. Because trisomics arise spontaneously at a low rate, some researchers used various chemical and physical agents to induce abnormal meiotic behaviour leading to the formation of *n+1* and other unbalanced meiotic products. Garber (1964) induced trisomy in *C. heterophylla* using colchicine. Rana (1965a) induced asynapsis in *Chrysanthemum* using high and low temperatures and Patil (1968) found trisomics in *Arachis hypogea* among progeny of plants whose inflorescences were treated with X-rays.

3. Asynaptic and desynaptic mutants

Recessive alleles at various loci in many, if not all, species cause asynapsis or desynapsis during meiosis (Beadle 1930; Prakken 1943; Soost 1951). In organisms homozygous for such mutant alleles, univalents are present in high frequencies at MI. As a result the distribution of chromosomes at AI is irregular and therefore leads to the formation of balanced(*n*) as well as unbalanced(*n-1*, *n+1*, etc) meiotic products. The primary trisomics and other types of aneuploids are the consequences of the union of appropriate balanced and unbalanced gametes. Koller (1938) found one trisomic plant among the progenies of an asynaptic mutant of

Pisum sativum. Goodspeed and Avery (1939) found 4.4% of the progeny of an asynaptic *N. sylvestris* mutant to be trisomics. Dyck and Rajhathy (1965) isolated six different primary trisomics from among the progeny of a desynaptic *A. strigosa* mutant. Palmer (1976) isolated two primary trisomics from an asynaptic mutant and one such aneuploid from a desynaptic mutant in *Glycine max* ($2n=2x=40$). Paria and Basak (1979) and Basak and Paria (1980) obtained a complete set of primary trisomics in jute, *Corchorus oblitoruis* ($2n=2x=14$), from among the progeny of an X-ray induced desynaptic mutant.

4. Haploids

If haploids are at least partially fertile these may produce trisomics as well as the other aneuploids. The requisite $n+1$ meiotic products can be generated in several ways depending on the behaviour of the univalents during meiosis. One way in which they can be produced is as follows: The chromatids of one univalent segregate to the same pole whereas the chromatids of the remaining univalents migrate to the opposite poles at AI. Absence of the second division therefore yields two unbalanced meiotic products, $n-1$ and $n+1$. Schertz (1963) recovered five trisomics from 394 progeny of a haploid *Sorghum vulgare* plant. Pochard (1968) isolated 49 trisomic plants from 2,500 progeny of a haploid of *Capsicum annuum* ($2n=2x=24$), representing the complete series of such aneuploids. Primary trisomics have also been derived from haploids in *Nicotiana tabacum* (Rao and Stokes 1963), *T. aestivum* (Sears 1939) and other polyploid species.

5. Plant tissue culture

Aneuploids can be generated from the *in vitro* cultures of plant tissues (Ronchi and Terzi 1988). Novak (1981) observed that some of the clones derived from somatic cell cultures of *Allium sativum* ($2n=2x=16$) were trisomics. Trisomics and other aneuploids have been observed among the progenies of plants generated from anther cultures in wheat and barley and somatic tissue cultures in oat, wheat, and triticale (Charmet *et al.* 1986).

6. Other sources

Primary trisomics can also arise from crosses between tetrasomics and diploids. Blakeslee and Avery (1938) obtained many such aneuploids in such crosses in *D. stramonium*. Primary trisomics also arise in the progenies of secondary, tertiary, and compensating trisomics as well as nonparental types due to nondisjunction in primary trisomics (Blakeslee and Avery 1938; Rick and Barton 1954; Chen and Grant 1968a; Khush 1973; Kuspira *et al.* 1986b). In *T. turgidum* Simeone *et al.*(1983) isolated 11 of the 14 primary trisomics in the first generation from crosses between tetrasomic or nullisomic-tetrasomic lines of *T. aestivum* cv Chinese spring and *T. turgidum*. One such trisomic was derived from a desynaptic mutant and two from a translocation heterozygote.

7. Sources of tetrasomics, secondary, tertiary, and compensating trisomics

Except for tetrasomics, these trisomic types were not the subject of investigations reported on in this thesis. Therefore we will only briefly allude to their mode of origin.

Telocentric chromosomes and isochromosomes arise by misdivision of univalents (Darlington 1939, 1940: Sears 1952). Tertiary chromosomes, with arms from two non-homologous chromosomes, are the products of reciprocal translocation (Belling 1925; Belling and Blakeslee 1926).

These trisomic types are derived from the same sources that give rise to primary trisomics (Blakeslee and Avery 1938; Goodspeed and Avery 1939; Khush and Rick 1966, 1967a and b, 1968a, 1969; Tsuchiya 1972; Singh *et al.* 1982a; Kumar *et al.* 1985; Shahla and Tsuchiya 1988).

C. Cytology of primary trisomics

1. Mitosis

The cytological identification of chromosomes at mitotic metaphase using standard staining and banding procedures as well as other techniques will be discussed in section IV. With rare exceptions, the mitotic behaviour of chromosomes in these aneuploids is similar to that in disomics in that each chromosome replicates its genetic material, auto-orients on the metaphase plate, divides equationally into chromatids which then segregate to opposite poles (Tsuchiya 1960; Meijer and Ahloowalia 1981).

2. Meiosis

In disomics, homologous chromosomes form bivalents(II) at diakinesis(D)-MI stages of meiosis. In primary trisomics on the other hand the chromosomes present in triplicate may exhibit one of 3 different associations: 3 univalents (Is), a bivalent plus a univalent (II+I), or a trivalent (III) (Belling and Blakeslee 1924; Einset 1943; Rick and Barton 1954). The presence of 3 univalents at D-MI is seldomly observed in meiocytes of different species. For example, in both *Z. mays* (Einset 1943) and *Pennisetum americanum* (Vari and Bhowal 1986) about 1% of the meiocytes possess three univalents. In meiocytes of *L. esculentum* (Rick and Barton 1954), *L. pedunculatus* (Chen and Grant 1968a) and other species such an association of the chromosomes in triplicate is not observed. The other two associations and their frequency of occurrence in a population of meiocytes depends on a variety of factors : species, size of chromosomes present in triplicate, modes of pairing of three homologues at pachytene, the location and frequency of occurrence of reciprocal exchanges and chiasmata within the pachytene associations, the extent of chiasmata terminalization and genetic factors (Einset 1943; Rick and Barton 1954; Dawson 1962). The longer chromosome pairs may exhibit a higher frequency of reciprocal exchanges and chiasmata than the shorter ones. Therefore the trisomics for these chromosomes may exhibit a higher frequency of trivalents. In *L. pedunculatus* II+Is are more common than IIIs being present in 73.3 and 14.7% of the meiocytes, respectively (Chen and Grant 1968a). In *Z. mays* (Einset 1943) and *H. spontaneum* (Tsuchiya 1960) the reverse is true for all trisomic types with average frequencies for II+Is and IIIs of 30.4 and 69.6% in corn and 11.2 and 88.8% in barley, respectively. In *L. esculentum* (Rick and Barton 1954) and *P. americanum* (Vari and Bhowal 1986) II+Is are more frequent than IIIs in some trisomics and the opposite is true in others, but on the average the latter associations occur more frequently with II+I and III frequencies of 38 and 40%, and 46.7 and 51.1% in tomatoes and pearl millet, respectively. However, in *Petunia axillaris* (Reddi and Padmaja 1982) and *P. americanum* (Singh *et al.* 1984; Vari and Bhowal 1986) the relationship between the length of the chromosome in triplicate and trivalent frequency is irrelevant. Vari and Bhowal (1986) suggested that the lack of correlation between chromosome length and trivalent formation might be due to the fact that chromosome pairing is under genetic control.

The manner in which the three homologues pair at pachytene as well as the number and position of reciprocal exchanges and chiasmata between them (Fig. 2A) yield four possible trivalent configurations at D (Belling and Blakeslee 1924; Dawson 1962; Benavente and Orellana 1984). These configurations can co-orient at MI in three different ways as shown in Fig. 2B (Dawson 1962; Kuspira *et al.* 1986b).The different trivalent configurations and MI co-orientations may or may not occur in different species. For example, Table 1 shows that chain and frying-pan type configurations are the most common with the former being the most frequent in *D. stramonium*, *L. esculentum*, *Pennisetum typhoides* and *P. americanum*. Triple-arc (Fig. 2A and B) configurations were not observed in *P. typhoides* (Manga 1976) and *P. americanum* (Vari and Bhowal 1986). The frying-pan configuration was the most common association in *H. spontaneum* indicating

Table 1. Types of trivalents at diakinesis - metaphase I and their frequencies in five plant species.

	Types of trivalents at D-MI (in percent)				References
	Chain	Frying-pan	Y-type	Triple-arc	
Datura stramonium	48.5	32.7	1.7	1.9	Belling and Blakeslee 1924
Lycopericum esculentum	53.1	41.2	4.7	1.0	Khush 1973
Hordeum spontaneum	40.6	54.1	0.4	4.9	Tsuchiya 1960
Pennisetum typhoides	90.0	6.7	3.3	-	Manga 1976
Pennisetum americanum	79.1	11.6	9.3	-	Vari and Bhowal 1986

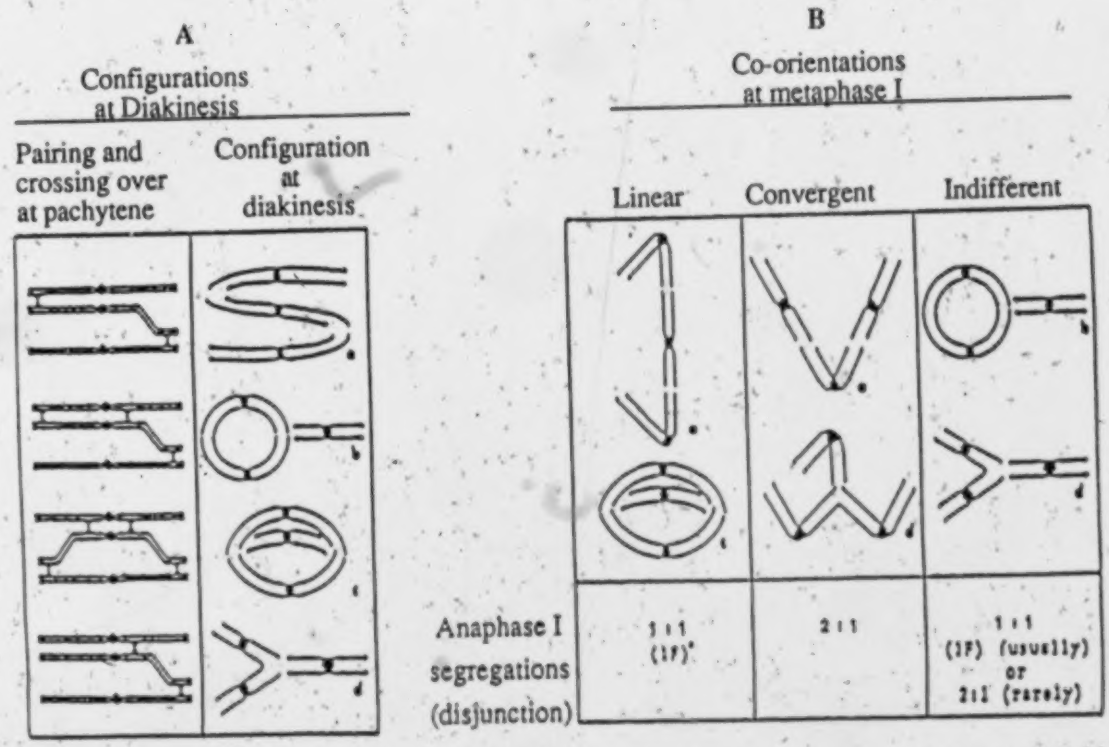

A
Configurations at Diakinesis
Pairing and crossing over at pachytene
Configuration at diakinesis
a
b
c
d
B
Co-orientations at metaphase I
Linear
Convergent
Indifferent
Anaphase I segregations (disjunction)
1 : 1 (1F)*
2 : 1
1 : 1 (1F) (usually) or 2 : 1 (rarely)

Fig. 2. A. Pairing and crossing over in a trivalent at pachytene and the resulting configurations at diakinesis.

B. Different co-orientations of a trivalent at metaphase I and the subsequent anaphase I segregations (the axes of the spindle are vertical).

*F. false univalents.

(From Kuspira *et al.* 1986b).

that the frequency of reciprocal exchanges per trivalent in barley may be higher than that in *Datura*, *Lycopersicum* and *Pennisetum*.

The segregation of chromosomes at AI is primarily determined by the orientation of their centromeres at MI. Univalents auto-orient at AI and either move to the poles at random, remain on the MI plate or misdivide (Sears 1952; Darlington 1957; Khush 1973). Chromosomes from bivalents segregate randomly in a regular (1:1) manner (Carrothers 1913; Balog 1979). The segregation of homologues from trivalent associations depends on the type of co-orientation at MI (Fig. 2A and B). From linear alignments the chromosomes segregate 1:1 with a false univalent behaving like a lagging univalent; chromosomes in an indifferent co-orientation usually behave in the same manner, occasionally segregating randomly in a 2:1 fashion; homologues in convergent co-orientations regularly segregate at random in a 2:1 fashion (Tsuchiya 1960: Dawson 1962; Kuspira *et al.* 1986b). A primary trisomic will yield *n+1* and *n* meiotic products in equal proportions only if trivalent associations that co-orient in a convergent manner at MI are formed in all of its meiocytes. In all other cases they will be formed in unequal proportions.

A detailed discussion of meiotic behaviour of other trisomic types can be found in various reviews (Khush and Rick 1966, 1967a and b, 1968b, 1969, Khush 1973, Singh and Tsuchiya 1981a).

D. Frequencies of trisomics in different crosses

Trisomic and diploid progeny are expected in equal proportions in reciprocal crosses between diploids and trisomics provided *n* and *n+1* gametes are produced and subsequently function in equal proportions on both the male and female sides in primary trisomics. On the other hand, self-fertilization of trisomics or crosses between two identical trisomics should produce diploid, trisomic, and tetrasomic(*2n+2*) progeny in a 1:2:1 ratio. However, theoretical expectations are seldom realized in the different plant species. The results of reciprocal crosses between $2n+1$ and $2n$ individuals of 13 diploid plant species, presented in Table 2, are typical of those in other such species. In the diploid plant species investigated, the average frequencies of trisomics in trisomic♀ x diploid♂ crosses are considerably less than 50%, with the exception of *Plantago lagopus* where the frequency is significantly high (70.6%, not shown in Table 2) (Sharma *et al.* 1985). Except for *L. pedunculatus* and *G. max*, in which the trisomics appear in the same range of frequencies in reciprocal crosses, the percentage of trisomic progeny is significantly lower in diploid♀ x trisomic♂ crosses than in the reciprocal matings in all other species. In species such as *Spinacea oleracea*, *H. spontaneum* and *L. perenne*, trisomics are not produced in $2n$♀ x $2n+1$♂ crosses. The frequency of trisomic progeny from self-fertilized trisomics (not shown in Table 2) is similar to that obtained in trisomic♀ x diploid♂ crosses in all species.

The extent of reduction in the expected frequency of trisomic progeny varies from one trisomic line to another within a species and from one species to another. Therefore, different causes can be expected for the reduction in different cases. The bases for this reduction in frequency of trisomic progeny in those species which have been investigated are as follows:

(i) Failure of the univalent to be included in meiotic products results in the formation of fewer than 50% $n+1$ meiotic products (Einset 1943; Koornneef and Van der Veen 1983).

(ii) Reduced viability of male and female $n+1$ meiotic products as well as the resultant gametophytes (Satina and Blakeslee 1937a and b; Khush *et al.* 1968a).

(iii) $n+1$ pollen grains are not as competent in effecting fertilization as the balanced (n) ones, either because they fail to germinate or they grow slower, or mature later (Buchholz and Blakeslee 1932; Tsuchiya 1960; Ramage 1965; Koornneef and Van der Veen 1983).

Table 2. Range of frequencies and average frequencies (in percent) of trisomic progeny in 13 intraspecific reciprocal crosses between $2n$ and $2n+1$ individuals.

	$2n+1$ ♀ x $2n$ ♂		$2n$ ♀ x $2n+1$ ♂		
Species	Range of frequencies of trisomics in different trisomic lines	Average frequencies of trisomics.	Range of frequencies of of trisomics in different trisomic lines	Average frequencies of trisomics	References
Nicotiana sylvestris	16.3 - 28.8	24.9	0.0 - 34.1	9.4	Goodspeed and Avery 1941
Hordeum spontaneum	9.8 - 27.2 (19.3 - 31.4)*	22.7 (25.9)*	0.0 - 0.0	0.0	Tsuchiya 1960
Secale cereale	16.0 - 39.3	26.5	0.0 - 2.6	0.8	Kamanoi and Jenkins 1962
Spinacea oleracea	3.9 - 34.5	17.3	0.0 - 0.0	0.0	Janick *et al.* 1959
Medicago spp.	5.5 - 24.7	14.0	0.0 - 17.4	3.5	Kasha and McLennan 1967
Lotus pedunculatus	2.9 - 21.6	8.6	3.1 - 26.9	8.9	Chen and Grant 1968b
Sorghum bicolor	0.0 - 20.9 (5.0 - 29.3)*	10.0 (15.2)	0.0 - 5.1	2.2	Liang 1979
Glycine max	34.2 - 45.2	39.3	22.3 - 43.7	35.7	Palmer 1976
Lolium perenne	12.0- 37.0	24.0	0.0 - 0.0	0.0	Meijer and Ahloowalia 1981
Oryza sativa	15.5 - 31.3	31.3	0.5 - 27.3	4.4	Khush *et al.* 1984
Pennisetum americanum	11.9 - 18.4 (13.8 - 23.8)*	13.9 (16.5)*	0.0 - 2.0	0.1	Singh *et al.* 1984
Arabidopsis thaliana	16.0 - 30.0 (15.5 - 43.2)*	24.2 (28.3)*	0.0 - 32.0	10.9	Kornneef and Van der Veen 1983
Datura stramonium	3.0 - 32.5*	22.1	-	-	Blakeslee and Avery 1938

*These ranges and averages are for progenies produced by self-fertilized trisomics.

(iv) Reduced viability of trisomic zygotes and embryos (Buchholz and Blakeslee 1932; Frost 1927; Tsuchiya 1960; Koornneef and Van der Veen 1983).

(v) Failure or delayed germination of trisomic seeds (Satina *et al.* 1938; Goodspeed and Avery 1939; Tsuchiya 1960; Vari and Bhowal 1986).

(vi) Reduced vigor of trisomic seedlings (Rick and Barton 1954; Liang 1979).

(vii) Effect of the genetic background (Rick and Notani 1961; Khush *et al.* 1984).

E. Occurrence of non-parental types of trisomics in the progenies of different primary trisomics.

In most, if not all species, primary trisomics produce related and non-parental types of trisomic progeny in low frequencies. Blakeslee and Avery (1938) and Avery *et al.* (1959) reported that each of the primary trisomics in *D. stramonium* produced related telotrisomic, as well as secondary and non-parental types of primary trisomic progeny. For example, related secondary trisomics were produced at the rate of 0.0 - 0.18%. Chen and Grant (1968b) showed that 0.6% of the progeny of primary trisomics in *L. pedunculatus* were unrelated trisomics in which the extra chromosome was not the same one as in the primary trisomic parent. Sharma *et al.* (1985) recovered several types of related and non-parental types of aneuploids among progenies of crosses between trisomic and disomic plants of *P. lagopus*. Simeone *et al.* (1983) obtained telotrisomics with an average frequency of 0.2% in crosses between trisomics for chromosomes 1A, 2A, and 5B and disomics in *T. turgidum*. Simeone *et al.* (1988) obtained trisomics for chromosome 7B among the progenies of trisomics for chromosomes 7A and 5B. Gwyn and Palmer (1989) obtained both double trisomic and tetrasomic progeny from crosses with five different primary trisomic lines of *G. max*. Similar findings have been reported in other species.

Blakeslee and Avery (1938) suggested that the presence of the extra chromosome might interfere with normal meiotic chromosome behaviour thereby resulting in the production of unrelated trisomics. However, it is neither clear nor has it been investigated as to why trisomics should increase the rate of spontaneous occurrence of unrelated trisomics and other aneuploids.

F. Phenotypes of trisomics

In diploid species of both plants and animals, it has been shown that the addition of a chromosome alters the genic balance and therefore leads to the expression of a multiple mutant phenotype (Bridges 1916, 1922; Blakeslee and Avery 1919; Blakeslee 1921b, 1922). Subsequent studies by Einset (1943), Rick and Barton (1954), Chen and Grant (1968a), and Khush *et al.* (1984) have shown that trisomy for a longer chromosome is more detrimental than trisomy for a shorter one in some species. The extent and direction of phenotypic shift in trisomics not only depends on the genic content of the chromosome present in triplicate but also on the ploidy of the organism (Khush 1973; Sears 1954; Kuspira 1988). In allohexaploids such as *T. aestivum* (Sears 1954) and *Avena sativa* (R. McGinnis, personal communication) the trisomics are phenotypically indistinguishable from each other and from disomics. Except for a few trisomics, the same is true of allotetraploids such as *N. tabacum* (Clausen and Goodspeed 1924) and *G. hirsutum* (Endrizzi *et al.* 1963; Kohel 1966).

Primary trisomics of diploid plant species fall into three groups on the basis of the morphology of their trisomics. In the first group, with the exception of one trisomic in each of *L. pedunculatus* (Chen and Grant 1968a) and *O. sativa* (Khush *et al.* 1984) all the trisomics of a vast number of species differ morphologically from each other and their diploid progenitors. Depending on the species, the primary trisomics can be distinguished either at the seedling or later stages of development. In *H. spontaneum* (Tsuchiya 1960), five of the seven primary trisomics were easily distinguishable at the early seedling stage from each other and the diploid sibs. The remaining two trisomic types were distinguished at later stages of ontogeny in this species. In *P. americanum*, the primary trisomics could be distinguished at both the seedling and later stages (Vari and Bhowal 1986). Fujigaki and Tsuchiya (1988) were able to classify all the primary trisomics of *S. cereale* at various stages of development and corroborate their classification cytologically. In *L. pedunculatus* the trisomics could be unequivocally distinguished only when they were fully grown (Chen and Grant 1968a). In addition to exhibiting specific phenotypic effects, these trisomics reduce vigor, fertility, longevity, and growth with the result that leaves, stems, and plants are smaller than those of the diploids. Moreover, these trisomics develop inflorescences, flower, and mature later than diploids.

In species of the second group including *Z. mays* (McClintock 1929; Rhoades and McClintock 1935) and *G. max* (Palmer 1976) only one or two trisomics can be identified morphologically. The others are indistinguishable from each other and the disomics. This has been attributed to the extensive duplication of genetic material that occurs in these organisms (Palmer 1976; Helentjaris *et al.* 1988; Wendel *et al.* 1989).

The trisomics in the species of the third group which includes *C. unguiculata* (Vasek 1956, 1961) and *C. heterophylla* (Garber 1964; Dhillon and Garber 1960) are vigorous, fertile and phenotypically indistinguishable from each other and the diploids. The tolerance of extensive aneuploidy in species of this group is probably also due to extensive duplication of genetic material.

G. Genetic and cytogenetic analyses using primary trisomics

Genetic and cytogenetic studies of F_2 and testcross (=backcross) progeny of heterozygous trisomics permits the assignment of genes to chromosomes present in triplicate in a given primary trisomic as first shown by Blakeslee *et al.* (1920).

Blakeslee and Farnham (1923), and Lesley (1926). Trisomics heterozygous for a gene locus on a chromosome present in duplicate will yield Mendelian F_2 (1:2:1 or 3:1) and testcross (1:1) phenotypic ratios. However, if the gene is located on the chromosome in triplicate the phenotypic ratios will deviate significantly from those expected on the basis of Mendelian inheritance. The extent of the deviation will depend on the meiotic behaviour of the chromosome in triplicate as well as the distance of the gene locus from the centromere. Genes close to the centromere will show chromosome segregation; those approximately 50 map units or further away from the centromere show random chromatid or maximum equational segregation whereas genes with intermediate locations yield phenotypic ratios somewhere between the two extremes (Burnham 1962; Kuspira 1988).

Species in which trisomic inheritance has been reported fall into two groups: (i) Those in which trisomics were only identifiable morphologically thus precluding the identification of the chromosome carrying the gene and its linkage group. These include *Datura* (Blakeslee and Farnham 1923; Blakeslee and Avery 1934), tomato (Lesley 1926, 1932), *Arabidopsis thaliana* (Koornneef and Van der Veen 1983), *A. majus* (Sampson *et al.* 1961), and *Sorghum bicolor* (Hanna and Schertz 1970). (ii) Those in which the trisomics were identifiable cytologically thus permitting identification of the chromosome carrying a given gene and its linkage group. These include maize (McClintock and Hill 1931; Rhoades and McClintock 1935), barley (Tsuchiya 1960), tomato (Rick and Barton 1954; Rick *et al.* 1964), spinach (Janick *et al.* 1959), rice (Khush *et al.* 1984), and potatoes (Wagenvoort 1982).

Primary trisomics have also been used to assign isozyme coding genes to chromosomes on the basis of increased levels of enzymatic activities in the aneuploids (Carlson 1972; Tanksley and Rick 1980; Sidhu *et al.* 1984; Ranjhan *et al.* 1988; Wu *et al.* 1988; Kaiser and Friedt 1989). For example, Tanksley and his colleagues (Tanksley 1983) have mapped more than 30 isozyme markers in tomato using primary trisomics.

The same logic has also been utilized in establishing linkage maps using cloned DNA sequences (Young *et al*. 1987; McCouch *et al*. 1988; Ellis and Cleary 1988). Young *et al*. (1987) and Tanksley (1987) established a genomic library of low copy number DNA sequences in tomato. Then groups of five to eight clones from this library were pooled and utilized as probes against a panel of trisomic DNAs which were restriction digested with the same enzyme used to construct the genomic library. The relative intensities of the hybridization bands in Southern blots were used to assign more than 70 single copy DNA sequences to specific chromosomes.

Whereas primary trisomics permit the overall assignment of genes and the linkage groups to specific chromosomes, telocentric and secondary trisomics permit determination of the chromosome arm location of each gene, the distance of each gene from the centromere and the orientation of the linkage group relative to the two ends of the chromosome (Khush 1973; Kuspira 1988). Investigations using telocentrics and secondary trisomics for the above stated purposes have been carried out most extensively in *D. stramonium* (Blakeslee 1924), tomato (Khush and Rick 1968b, 1969), maize (Rhoades 1933, 1936), and barley (Tsuchiya 1960; Shahla and Tsuchiya 1988).

IV. Cytological and molecular identification of chromosomes and trisomics

A. Standard methods

1. Historical perspective

Prior to the 1920s cytological studies were carried out on biological tissues that were embedded in paraffin, sectioned, and stained (Wilson 1925; Darlington 1937). The methods that were in vogue were not sufficiently refined to allow for the detection of such gross morphological features as centromeres, secondary constrictions, and satellites of chromosomes. They merely facilitated the determination of chromosome numbers and permitted detection of approximate size differences among the chromosomes in somatic and meiotic cells of many eukaryotic species. For example, in the genus *Triticum*, Sakamura (1918) showed that the somatic cells of the species *monococcum*, *turgidum*, and *aestivum* possesed 14, 28, and 42 similar-sized chromosomes, respectively.

During the 1920s and 1930s innovations were introduced which facilitated cytological and karyotypic analyses. In 1921, Belling described a technique for studying meiosis in plant species that involved the squashing of anthers. This method permitted the separation of PMCs and facilitated the spreading of their chromosomes. In 1929, Kagawa, working with *Triticum* and *Aegilops* species, demonstrated that treatment with chloral hydrate before fixing and staining the cells shortened the chromosomes. This makes it easier to separate them and study their gross morphological features: centromeres, secondary constrictions, and satellites. Pre-treatment with other agents such as alpha bromonaphthalene (Schmuck and Kostoff 1935), colchicine (O'Mara 1939), paradichlorobenzene (Meyers 1945) and cold water (Hill and Myers 1945) also permitted identification of these chromosomal substructures. By the early 1940s the squashing technique, concomitant with appropriate modifications and pretreatments, completely replaced the method of microtome sectioning of tissues in chromosome studies using somatic and meiotic tissues of most species (Hillary 1938; Aase 1935; O'Mara 1939).

The squash technique, with appropriate modifications, was also used successfully in chromosome studies of insects, amphibians, and other animals (White 1954), excluding mammals (Hsu 1979). Mammalian cytology had to await the innovations of hypotonic solutions (Hsu 1952), *in vitro* culturing of tissues and cells (Hsu and Pomerat 1953), and their colchicine pretreatment (Hsu and Pomerat 1953) for obtaining good spreads of somatic chromosome complements. Using these innovative procedures the chromosome number in man was first correctly determined to be $2n=46$ by both Tjio and Levan, and Ford and Hamerton in 1956. In the ensuing years this technique, combined with the use of phytohemagglutinin (Nowell 1960) to stimulate cell divisions, was applied to karyotyping euploids, aneuploids and individuals with chromosomal abnormalities in numerous mammalian species (see Hsu and Benirschke 1967-1977). The various techniques that have been used to this day for karyotyping hundreds of species using gross chromosomal morphological features are detailed in La Cour (1947), Darlington and La Cour (1960), Sharma and Sharma (1965), and Haskell and Wills (1968).

2. Standard somatic karyotypes in plant species

In plant species, such as maize (Rhoades and McClintock 1935), tomato (Rick and Barton 1954; Rick *et al.* 1964) and rice (Chu 1967; Kurata and Omura 1978) the individual chromosomes (and therefore trisomics) can not be identified in somatic cells using standard staining procedures because the chromosomes are either too small and/or similar in morphology. In most of the plant species, however, at least a few of the chromosomes and trisomics can be identified in standard somatic karyotypes. For example, in barley (Tsuchiya 1960), and *Petunia axillaris* (Reddi and Padmaja 1982), three of the chromosome pairs and trisomics can be identified using standard procedures. In beets (Romagosa *et al.* 1986) eight of nine chromosomes and trisomics can be identified in the standard fashion and in *A. strigosa*, Rajhathy (1975) was able to distinguish all chromosomes and identify all trisomics from standard somatic karyotypes.

Although numerous karyotypic studies have been carried out since 1939 in several species of the genus *Triticum*, only the species *T. monococcum*(AA), *T. turgidum*(AABB), and *T. aestivum*(AABBDD) will be reviewed here. Studies by Camara (1943), Coucoli and Skorda (1966), Giorgi and Bozzini (1969b) and Kerby and Kuspira (1988) have shown that there are 14 similar chromosomes in the diploid complement of *T. monococcum* ; one ST pair, two M pairs, and four SM pairs. Depending on the accession line studied, either one or two chromosome pairs were found to possess satellites (Camara 1943; Riley *et al.* 1958; Coucoli and Skorda 1966). The karyotype of *T. turgidum* consists of two SAT pairs, two ST pairs, seven SM pairs, and three M pairs of chromosomes (Giorgi and Bozzini 1969a; Kerby and Kuspira 1988). Since the A genome has the chromosome constitution given above, the karyotype of the B genome in *T. turgidum* must consist of two SAT pairs, one ST pair, three SM pairs, and one M pair of homologues. Two of these chromosome pairs possess large satellites (Pathak 1940; Riley *et al.* 1958) which belong to the B genome (Okamoto 1957). The satellites in the A genome in *T. turgidum* are suppressed (Riley *et al.* 1958). At most, four to six of the chromosomes in the somatic complement of this species can be distinguished using standard procedures.

Depending on the genotype studied, either one (Sears 1954), two (Morrison 1953), three (Pathak 1940; Camara 1943), or four (Kagawa 1929; Schulz-Schaeffer and Haun 1961) satellited chromosomes are observed in *T. aestivum*. These belong to the B and D genome (Sears 1954; Schulz-Schaeffer and Haun 1961). Camara (1943), Morrison (1953), Sears (1954,1958), Schulz-Schaeffer and Haun (1961), and Gill (1987) have shown that the chromosomes in the somatic complement of bread wheat are similar in size. For example, Gill (1987) reported that they range in length from 8.4*u*m for chromosome 1D to 13.8 *u*m for chromosome 3D. The latter observations also show that (i) except for the chromosome 4A pair, all other pairs in homoeologous groups 1, 4, and 5 are highly heterobrachial (ST), (ii) except for the chromosome 7B pair, all other pairs in homoeologous groups 6 and 7 are M, and (iii) chromosome pairs in homoeologous groups 2 and 3 as well as chromosome pairs 4A and 7B are SM. Even in the best somatic metaphase spreads, only a limited number of chromosomes and chromosome pairs can be distinguished by standard methods.

3. Chromosome identification during meiosis

Standard staining procedures render nucleoli to be clearly visible at pachytene stage and permit the detection of chromosomes that carry NORs. Moreover, in corn (Rhoades and McClintock 1935), tomato (Rick and Barton 1954), and rice (Khush *et al.* 1984) it is possible to identify each univalent, bivalent, and multivalent association on the basis of its length and chromomere pattern during pachytene. Thus a chromosome in triplicate in these species is easily identified by examination of the trivalent configuration at pachytene using standard staining techniques.

B. Banding techniques

In almost all species the usefulness of standard staining procedures, however, has been limited. Although they have facilitated the ascertainment of chromosome numbers and gross morphological features of chromosomes, they have not permitted an accurate and unequivocal identification of all the chromosomes, and therefore the aneuploids, of a species. An exhaustive analysis of the karyotype requires the use of staining procedures that can reveal each chromosome as a specific, unique, and constant pattern of alternating dark and light banding regions, topologically equivalent to the bands in the polytene chromosomes in salivary gland cells of *D. melanogaster*.

Darlington and La Cour (1940) demonstrated that with cold treatment of somatic cells of *Trillium erectum* some regions of chromosomes revealed unique patterns by appearing thinner and less intensely stained than the rest of the chromosomes. The utilization of fluorescent and other dyes together with various modifications in pretreatment of cytological material in the late 1960s heralded a new era of cytogenetics. New and reliable staining procedures were introduced, each of which was capable of revealing a unique "banding pattern" of the chromosomes of a given species. By 1972 the application of one or another of five major banding techniques (Q, G, R, C, and N) for the purpose of karyotypic analysis came into vogue. These have led to a more precise cytogenetic and phylogenetic analysis of various eukaryotes.

1. Q-banding

Caspersson and his colleagues in 1968 were first to demonstrate that fluorescent dyes such as quinacrine and quinacrine mustard bind preferentially to certain regions of normal mitoic chromosomes of *Cricetulus griseus*, *V. faba* and *T. erectum*. As a result, unique patterns of brightly fluorescent regions alternating with non-fluorescent (dark) regions were produced in each chromosome. Weisblum and de Haseth (1972) and Burkholder (1988) have shown that fluorescent dyes interact with AT base pairs and those regions of DNA that are sufficiently AT-rich (70 - 100%) fluoresce and appear as bright bands (Q bands). Q-banding permits an identification of all the chromosomes and their homologoues in most species. For example, in man all 23 pairs of homologous chromosomes can be distinghished on the basis of their Q-banding patterns (Caspersson *et al.* 1971). In *Scilla sibirica* all eight chromosome pairs can be identified (Caspersson *et al.* 1969). Q-banding does not require any pretreatment and is the simplest of all the banding methods. Compared to other banding techniques, it has several disadvantages; the fluorescent bands are not permanent; the technique requires the use of ultravilolet light, and does not stain ends of chromosomes. As a consequence Q-banding has been used to a limited extent, and since the late 1970s (Pinkel *et al.* 1988) has been largely replaced by other banding methods. In plants, Q-banding studies have been limited to a few in *Trillium*, *Scilla*, *Allium*, *Crepis*, *Lilium*, *Secale*, and *Vicia* (Caspersson *et al.* 1969; Vosa and Marchi 1972a; Kongsuwan and Smyth 1977; Schweizer 1980; Rowland 1981).

2. G-banding

In 1971, Drets and Shaw, Patil *et al.*, Seabright, and Sumner *et al.* independently developed a protocol for animal species whereby each chromosome segment and chromosome revealed a unique pattern of bands. Each of the protocols, by using a variety of treatments before fixing chromosomes and staining with Giemsa, yields a banding pattern in normal mitotic chromosomes that is similar to the one revealed by the Q-banding technique. The dark regions are the topological equivalents to Q-bands and are called G-bands whereas the light regions are equivalent to the nonfluorescent dark ones revealed with the use of fluorescent dyes (Drets and Shaw 1971; Dutrillaux and Lejeune 1975). Application of G-banding methods to prophase and prometaphase chromosomes in animals reveals a larger number of bands than at metaphase which permits more precise karyotyping and cytogenetic analysis (Yunis 1981; Iannuzzi 1990).

The basis for G-banding is currently unknown. One plausible explanation is that of Comings (1978) who postulated that prophase and metaphase chromosomes contain a basic chromomeric structure that can be enhanced. This enhancement occurs by inducing some rearrangement of the fibers away from the light bands toward the G-bands, possibly some extraction of light-band DNA with denatured nonhistone proteins, followed by the marked enhancement of this pattern through the ability of thiazin dyes in Giemsa to side stack on available DNA. Sumner (1982) and Burkholder (1988) have proposed alternate mechanisms.

Although the technique has been attempted in many plant species, G-bands have been generated in the chromosomes of only a few species: *Tulipa gesneriana* (Filion and Blakey 1979), *Pinus resinosa* (Drewry 1982), and *Vicia hajastana* (Wang and Kao 1988). The failure to produce G-bands in the chromosomes of most plant species, including those in the *Triticeae*, has been attributed to the increased condensation of the plant chromosomes (Greilhuber 1977; Drewry 1982). Anderson *et al.* (1982), however, failed to show consistent differences in the degree of compaction, based on measurements of lengths and volumes of chromosomes from several plant and animal species. Wang and Kao (1988) demonstrated that improper pretreatment of plant chromosomes alters the organization of their chemical constituents and renders them unresponsive to the G-banding procedure.

3. Reverse(R)-banding

This banding technique was developed by Dutrillaux and Lejeune in 1971. Mild denaturation by heat and subsequent staining of chromosomes with Giemsa or a fluorochrome dye reveals a banding pattern that is the reverse of the patterns produced by the G- and Q-banding methods (Bobrow *et al.* 1972; Comings 1973; Dutrillaux *et al.* 1973). Specifically, if the chromosomes are stained with Giemsa, the dark bands (R bands) produced with this technique are equivalent to the light bands produced by the G-banding technique and vice-versa (Dutrillaux and Lejeune 1971, 1975). If a flurochrome dye such as acricine orange or olivomycin is used, fluorescent R-banding is the reverse of Q-banding in that the R-bands fluoresce bright green and the non-R-bands show a faint red color (Schweizer 1976; Lin *et al.* 1980; Schmid and Guttenback 1988). R-banding is particularly useful in the detection of structural rearrangements involving ends of chromosomes in that it stains telomeres as T-bands (Dutrillaux *et al.* 1973).

R-bands have been detected in only few plant species e.g., *S. sibirica*, *V. fava*, *Allium* spp., none of which belong to the *Triticeae* tribe (Schweizer 1980; Deumling and Greilhuber 1982; Loidl 1983). Moreover, since the R-bands in these species are few in number and faint, they have not been used for karyotyping and cytogenetic studies.

R-bands can be produced by GC-specific fluorochromes (Schweizer 1976; Van de Sande *et al.* 1977; Holmquist *et al.* 1982), although the mechanism of R-banding is unknown (Burkholder 1988). The mechanism proposed by Comings (1978) may also explain R-banding if the DNA and proteins in the G- and R-bands are selectively denatured under different conditions of pH, salt concentrations, and temperature.

4. C-banding

Pardue and Gall (1970) and Jones (1970) independently demonstrated a procedure which with stringent treatment of chromosomes prior to fixation and staining with Giemsa, stained only the regions of constitutive heterochromatin in chromosomes of *Mus musculus*. These regions, now referred to as C-bands, were observed to be proximal to the centromeres of all the chromosomes in this species and have since been demonstrated in chromosomes of the guinea pig (Yasmineh and Yunis 1975), *Drosophila* spp. (Gall and Atherton 1974; Brutlag *et al.* 1977), *Rattus rattus* (Yosida and Sagai 1975) and many other animal species. Constitutive heterochromatin usually appears as "satellite-DNA" when nuclear chromosomal DNA is fragmented and centrifuged (Kit 1961). It consists of short, highly repeated base pair sequences in tandem (Southern 1970; Corneo *et al.* 1970; Gall and Atherton 1974; Brutlag *et al.* 1977) in one or more regions of all or most chromosomes in most species. Arrighi and Hsu (1971) showed that C-bands are located next to the centromeres of each chromosome, next to the secondary constrictions of chromosomes 1, 9, and 16 as well as the satellites of acrocentric chromosomes in man. With few exceptions, constitutive heterochromatin in animal species reveals a consistent pattern of distribution. Therefore, C-banding in animal species does not correspond to a banding pattern in a strict sense. Its application in these species is limited because it does not allow precise recognition of individual chromosomes.

Several lines of evidence indicate that the production of C-banding is due to the extraction of non-C-band DNA and denaturation of proteins in these regions. The DNA in constitutive heterochromatin is resistant to extraction, remains within the chromosomes and is therefore stainable by Giemsa (Pathak and Arrighi 1973; Dille *et al.* 1987; Burkholder 1988).

Since the initial studies in *V. fava* by Vosa and Marchi in 1972, chromosomes of many species of *Aegilops*, *Agropyron*, *Elymus*, *Hordeum*, *Secale*, and *Triticum* (Gill and Kimber 1974 a and b; Linde-Laursen 1975; Vosa 1976; Gerlach and Peacock 1980; Singh and Tsuchiya 1981b; Seal 1982; Teoh and Hutchinson 1983; Endo 1986; Morris and Gill 1987) and other plant species (Linde-Laursen *et al.* 1980; Loidl 1983) have revealed C-bands. These studies show that there is a fundamental difference in the distribution of constitutive heterochromatin within chromosomes of animals and plants. C-bands in the chromosomes of plants can be located at various sites including the regions they characteristically occupy in the chromosomes of animals. Moreover, in most of the plant species e.g., *Allium carinatum* (Loidl 1983), *Hordeum* spp. (Linde-Laursen *et al.* 1980) and *Agropyron elongatum* (Endo and Gill 1984a), some of the chromosomes do not reveal C-bands next to their centromeres. Thus, in many plant species a unique C-banding pattern occurs in each arm of each chromosome in the genome. This allows individual chromosomes in the somatic cells to be identified on the basis of their patterns (loc. cit.).

Gill and Kimber (1974a) published the first report on C-banding patterns of chromosomes of *T. aestivum*. Despite the efforts of many investigators in the interim, Endo (1986), using an improved C-banding technique, was able to unequivocally identify all 21 chromosomes in the genome of cvs. Chinese Spring and Norin 61 of *T. aestivum*. Fig. 3 is a composite karyotype which shows the C-banding patterns of the 21 chromosomes and the 42 telocentrics of *T. aestivum* cv. Chinese Spring. These banding patterns are currently accepted as standard patterns for the chromosomes of common wheat (Gill 1987; Gill *et al.* 1988). Ferrer *et al.* (1984) applied the C-banding protocol to the study of chromosomes in meiocytes and clearly identified nine of the 21 chromosome pairs.

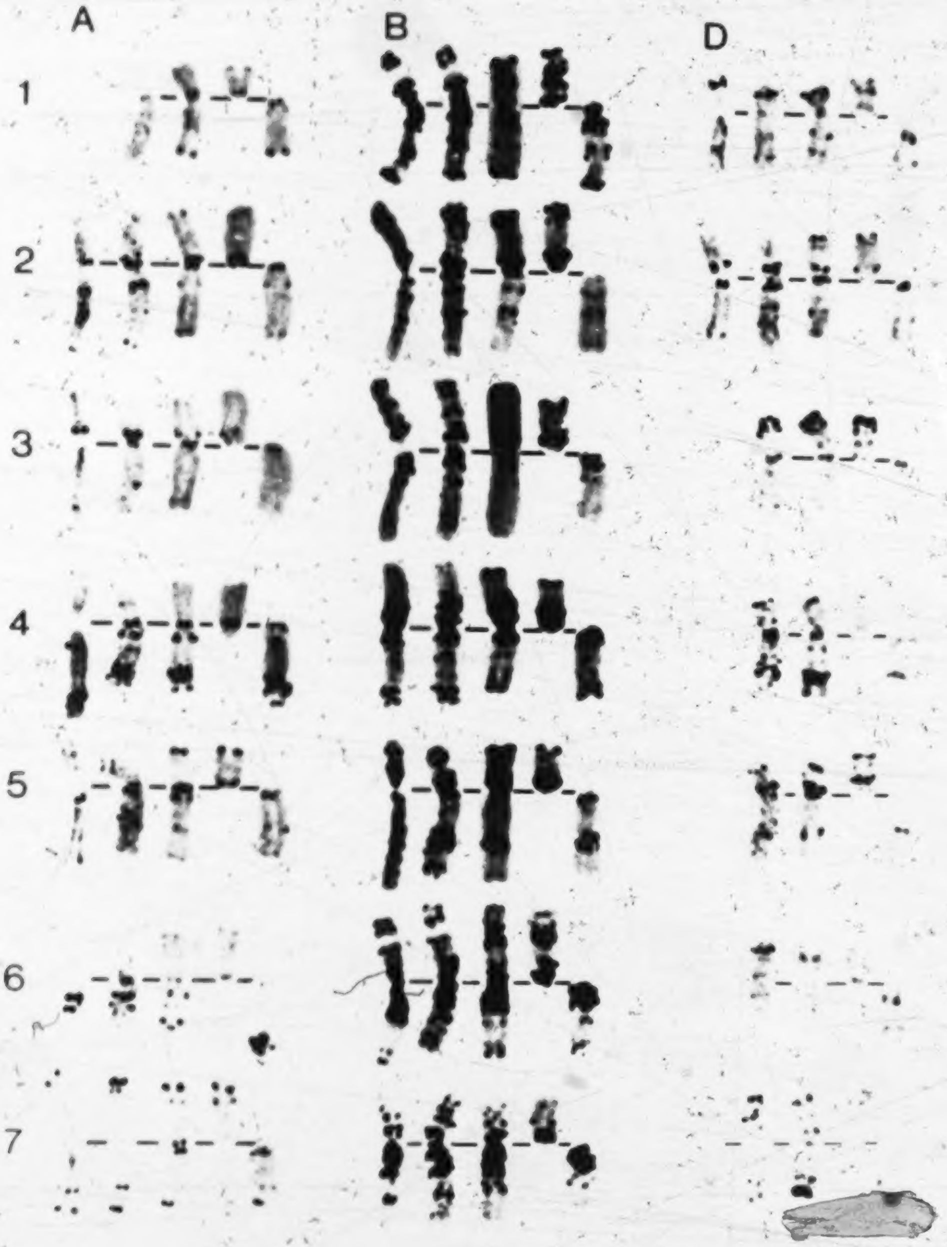
Except for the work of Simeone *et al.* (1988), the efforts of other investigators (Zurabishibili *et al.* 1978; Seal 1982; Lukaszewski and Gustafson 1983) to identify the A and B genomes of *T. turgidum* on the basis of their C-banding patterns have been inconsistent. Simeone *et al.* (1988) reported C-banding patterns for chromosomes of *T. turgidum* that were equivalent to those of their homologues in the A and B genomes of *T. aestivum* and therefore permitted their unequivocal identification. Shang *et al.* (1989), used the HKG (HCl-KOH-Giemsa) method to produce banding patterns in chromosomes of *T. turgidum* that were in part similar to their C-banding patterns, thus allowing identification of some of the chromosomes.

C-banding of the chromosomes of *T. monococcum* has been reported by both Gill and Kimber (1974a) and Kuz'menko *et al.* (1987). The banding patterns of the chromosomes in these two investigations were partially disimilar. Moreover, the C-banding patterns of some of the chromosomes in these publications were different from those for the A-genome chromosomes in *T. aestivum*, thus

Fig. 3. N- and C-bands in all 21 chromosomes and their telocentrics of *T. aestivum*, cv. Chinese Spring. First column: N-bands of 16 chromosomes*. Second column: C-bands of all 21 chromosomes (Endo 1986). Third column: C-bands of all 21 chromosomes (B. Friebe 1989 unpublished). Fourth column: C-bands of short arm telocentrics of all 21 chromosomes (B. Friebe 1989 unpublished). Fifth column: C-bands of long arm telocentrics of all 21 chromosomes (B. Friebe 1989 unpublished). This figure was kindly provided by Dr. B. Friebe.

*The remaining 5 chromosomes do not show N-bands.

(Magnification : 1500 X)


A
B
D
1
2
3
4
5
6
7

precluding their identification. Using the HKG method, Shang *et al.* (1988 and 1989) reported banding patterns for the chromosomes of *T. monococcum*, partially, resembled their C-banding patterns. Although the banding patterns revealed by the HKG method rendered some of the chromosomes distinguishable from the others, they did not allow for their unequivocal identification.

C-banding has been used in the identification of aneuploids (Linde-Laursen 1978b, 1982; Zeller *et al.* 1987), translocations and other structural rearrangements (Gill and Kimber 1977; Lukaszewski and Gustafson 1983; Lapitan *et al.* 1984) and the precise physical mapping of genes (Kota and Dvořák 1986; Jampates and Dvořák 1986). C-banding analysis of *durum-timopheevi* and *durum-speltoides* hybrids by Chen and Gill (1983) has supported Dvořák's suggestion (1983) that chromosomes 4A and 4B should be reassigned to the B and A genomes, respectively. Moreover, C-banding has clarified and further substantiated phylogenetic conclusions based on chromosome pairing in interspecific and intergeneric hybrids (Gill and Kimber 1974a; Hutchinson and Miller 1982; Chen and Gill 1983; Morris and Gill 1987).

5. N-banding

In 1973 Matsui and Sasaki developed a technique they called N-banding, which selectively stained NORs in the chromosomes of mammalian species. Funaki *et al.* (1975) improved this procedure and demonstrated that N-bands were confined to the NORs of the chromosomes of 27 eukaryotic species that thay studied. Faust and Vogel (1974) and Pimpinelli *et al.* (1976) observed that the bands obtained with this procedure are not NOR-specific in *D. melanogaster* and the mammalian species studied. Nevertheless, these non-NOR bands were and continue to be referred to as N-bands. Using the method of Funaki *et al.* (1975), with slight modifications, Gerlach (1977) and Jewell (1979), working with *Triticum* and *Aegilops* species respectively, clearly demonstrated that N-bands do not necessarily correspond to NORs. Moreover, at least some of the chromosomes in the species analyzed had unique N-banding patterns, permitting their identification. Gerlach (1977) identified nine of the 21 chromosomes of common wheat on the basis of their N-banding patterns. Subsequently, Endo and Gill (1984a) identified 16 of the 21 chromosomes of common wheat, including five in the A genome, using an improved N-banding protocol. Jewell (1979) identified all 14 chromosomes of *Aegilops variabilis* on the basis of their N-banding patterns. The technique has also been used to identify chromosomes in barley (Singh and Tsuchiya 1982b), rye (Jewell 1981; Schlegel and Gill 1984), lentils (Mehra *et al.* 1986) and *Elymus* spp. (Morris and Gill 1987). N-banding has also been used to identify various types of aneuploids (Singh and Tsuchiya 1982b; Zeller *et al.* 1987), alien addition and substitution lines (Islam 1980), and translocations and deletions (Jewell 1978). It should be noted that N-banding has been attempted in *T. monococcum* by B.S. Gill (personal communication) and in our laboratory without success. Why this should be, since some of the A genome chromosomes in *T. aestivum* contain N-bands, is unknown.

Gerlach (1977), and subsequently others, noted that many of the N-bands occupy the same positions as C-bands, implying that the N-banding technique, like the C-banding one, identifies constitutive heterochromatin and that at least two classes of heterochromatin occur in wheat, rye and other species. This was confirmed by Schlegel and Gill (1984) and Endo and Gill (1984a). Some heterochromatic regions in each chromosome stain positively using both C- and N-banding procedures. These regions are referred to as C^+N^+ bands. Other such regions stain positively only with C-banding techniques. These heterochromatic segments are called C^+N^- bands. Schlegel and Gill (1984) have shown that only N-bands (C^+N^+ bands) possess multiple copies of the $(GAA)_n(GAG)_n$ sequence DNA. The base pair sequences in C^+N^- bands have not been identified.

Gill (1987) and Gill *et al.* (1988) have proposed banding nomenclatures for the chromosomes of *T. aestivum* cv. Chinese Spring.

C. Identification of NORs and Nucleoli

Nucleolus organizer regions (NORs) are the sites of rRNA genes in the chromosomes of animal (Ritossa and Spiegelman 1965; Wallace and Birnstiel 1966; Henderson *et al.* 1972, 1974) and plant species (Phillips *et al.* 1971; Flavell and O'Dell 1976; Hutchinson and Miller 1982). Methods have been developed for the selective staining of these chromosomal regions both in animals (Goodpasture and Bloom 1975; Howell *et al.* 1975; Verma and Babu 1984) and plants (Hizume *et al.* 1980; Lacadena *et al.* 1984; Mehra *et al.* 1985; Cunado *et al.* 1986). The Ag-As (Ammoniacal silver) method selectively stains those sites on chromosomes which correspond exactly to regions that can be detected by *in situ* hybridization with rDNA probes (Howell *et al.* 1975; Miller *et al.* 1976a and b). It seems that this procedure stains only the NORs that are functionally active during the preceding interphase (Howell 1977; Schmiady *et al* 1979). There is evidence to suggest acidic or nonhistone proteins associated with the rDNA regions are responsible for the selective staining of NORs (Howell *et al* 1975; Wang and Juurlink 1979; Howell 1985).

D. *In situ* hybridization and its application to wheat cytogenetics

Gall and Pardue (1969) and John *et al.* (1969) independently reported a procedure that facilitated the cytological detection of hybrid nucleic acid regions. This technique involves the annealing of radioactively labelled nucleic acid sequences to cytological (chromosomal) preparations *in situ* (on slides) and subsequent detection of the hybrid regions by autoradiography. Specific DNA sequences in the chromosomes of animal and plant species have been localized with this technique. These include some highly repetitive short base-pair sequences in the chromosomes of animals (Pardue and Gall 1970; Brutlag *et al.* 1977) and plants (Gerlach and Peacock 1980; Appels and McIntyre 1985; Ganal *et al.* 1988; Lapitan *et al.* 1989), moderately repeated sequences such as rRNA genes in the chromosomes of animals (Wimber and Steffensen 1970, 1973; Henderson *et al.* 1972) and plants (Wimber *et al.* 1974; Gerlach and Bedbrook 1979; Mascia *et al.* 1981; Clark *et al.* 1989), and some single copy genes in animals (Harper and Saunders 1981; Henderson 1982; Olsen *et al.* 1989) and plants (Ambros *et al.* 1986; Huang *et al.* 1988).

Gerlach and Peacock (1980) isolated a highly repetitive DNA sequence from *T. aestivum* cv. Chinese Spring and hybridized it to cytological preparations of *T. aestivum*, *T. dicoccoides*, *T. monococcum*, and *Ae. squarrosa*. A number of heavily labelled chromosomes were observed in the preparations of *T. aestivum* and *T. dicoccoides*, but not in those of *T. monococcum* and *Ae. squrrosa*. On the basis of these results the authors concluded that most of the heavily labelled chromosomes belong to the B genome. C-banding studies by Endo (1986) and Gill (1987) and N-banding reports by Endo and Gill (1984b) support this conclusion. Peacock *et al.* (1981) demonstrated that the DNA sequence is composed of repeated $(GAA)_n$ and $(GAG)_n$ units. Rayburn and Gill (1985) showed that the major C- and N-bands correspond to sites which contain this satellite sequence. *In situ* hybridization studies with a highly repetitive D-genome specific DNA sequence isolated from *Ae. squarrosa* were used by Rayburn and Gill (1986) to identify D-genome chromosomes in hexaploid wheat.

At least four pairs of chromosomes (1A, 1B, 5D, and 6B) of *T. aestivum* contain NORs (Crosby 1957; Darvey and Driscoll 1972). If NORs are the sites of ribosomal RNA (rRNA) genes, then all these chromosomes should be expected to possess clusters of rRNA genes. Flavell and Smith (1974a, 1974b) and Flavell and

O'Dell (1976, 1979) showed that in hexaploid wheat a large proportion of the rRNA genes are on chromosomes 1B and 6B, with only a small proportion of the genes residing on chromosomes 1A and 5D. Gerlach and Bedbrook (1979) cloned the 18S+26S rRNA genes of *T. aestivum* into a bacterial plasmid and showed that the probe derived from this clone hybridized to regions on chromosomes 1B and 6B. Miller *et al.* (1980) showed that the same rDNA probe hybridized to minor NORs on chromosomes 1A and 5D in bread wheat. A similar approach with a different rDNA probe enabled Appels *et al.* (1980) to confirm the location of rRNA genes on chromosomes 1B, 5D, and 6B only. They speculated that too low a level of rRNA genes on chromosome 1A may have precluded their detection by *in situ* hybridization experiments. *In situ* hybridization experiments in *T. turgidum* and *T. timopheevi* with rDNA probes confirmed the assignment of rDNA loci to chromosomes 1B and 6B (Appels and Dvořák 1982; Dvořák and Appels 1982). Miller *et al.* (1983) showed that, in *T. urartu*, a labelled rDNA probe hybridized *in situ* to a region on chromosome 5A that corresponds to the NOR. Some genotypes of *T. urartu*, and other diploid wheats, have been shown to have two chromosome pairs with nucleolus organizers (Gerlach *et al.* 1980). The second NOR must, by deduction, be located on chromosome 1A. Frankel *et al.* (1988), using a synthetic tetraploid AABB and a ^{3}H-labelled rDNA probe, clearly demonstrated that the NORs of two pairs of A-genome chromosomes were labelled after *in situ* hybridization. Apart from one being more heavily labelled than the other, the two chromosome pairs (1A and 5A) could not be distinguished cytologically.

Information on the location of 5S rRNA genes in wheat species is scanty. Appels *et al.* (1980) localized the 5S rRNA gene cluster to chromosome 1B of *T. aestivum*, at a site distinct from and distal to the NOR region. Kota and Dvořák (1986) mapped these genes to a single site on the *p* arm of chromosome 5B, using a line with a spontaneous deletion. Lassner and Dvořák (1985) and Kota and Dvořák

(1986) suggested that chromosomes 5A and 5D may also carry the genes for 5S rRNA. Scoles *et al.* (1987), using cloned 5S rDNA sequences obtained unequivocal evidence for the presence of 5S rRNA genes on chromosomes 1B, 1D, and 5B in *T. aestivum*. Studies by Dvořák *et al.* (1989) have shown that chromosomes 1A and 5A of *T. monococcum* var. *aegilopoides* carry 5S rRNA genes. Moreover, they indicate that the 5S rDNA on chromosome 1B is linked to the Nor-B1 locus.

E. Production of molecular probes

Molecular probes, including those used in this study, are derived from cloned recombinant DNA molecules e.g., plasmids such as pBR 322 with cDNA genes. These molecules are generated using restriction and other DNA modifying enzymes and then cloned in a proper host (Mertz and Davis 1972; Watson *et al.* 1983). Protocols for generating recombinant DNA molecules, cloning them, excising the relevant DNA fragment e.g., 5S rDNA genes from these molecules and subsequent use in *in situ* hybridization and other experiments are detailed in Watson *et al.* (1983) and Winnacker (1987). The procedures used in the cereals are given by Gerlach and Bedbrook (1979) and Lawrence and Appels (1986).

F. Restriction fragment length polymorphism (RFLP) for chromosome identification

RFLP markers are currently being extensively used in genetic mapping (Wyman and White 1980, Helentjaris 1987, White and Lalouel 1988) and dertermining genome homologies among crop species (Bonierbale *et al.* 1988, Sharp *et al.* 1989). They have also been used in the identification of the critical chromosomes in aneuploid plants in the tomoto (Young *et al.* 1987) and bread wheat (Gale *et al.* 1988).

Sharp *et al.* (1989) isolated specific RFLP probes for each arm of the seven chromosomes of the homoeologous genomes of *Triticum*. These 14 homoeologous probes permit the identification of all chromosomes in each genome of the diploid and polyploid wheats.

MATERIALS and METHODS

I. Materials

A. Parental strains

Four true breeding diploid accessions of *Triticum monococcum* L., hornemannaii (Assyrischer Thonda) (line 89), PI 330551 (line 68), PI 306542 (line 253) and PI 272519 (line 278) were used in producing the trisomics for this investigation. The accessions were obtained by Dr. J. Kuspira from Dr. D.H. Smith, Jr., United States Department of Agriculture, Beltsville Agriculture Research Center, Beltsville, Maryland, U.S.A. Their ploidy was confirmed by determining the number of chromosomes in meiocytes (microsporocytes). A 29-chromosome segregant (which possessed 13 pairs of *T. turgidum* chromosomes plus a 4A chromosome from this species and a pair of 4A chromosomes from *T. monococcum* var. *bocoticum*) was used to identify one of the chromosomes of *T. monococcum*. This segregant was a derivative of an F_3 generation plant containing 29-chromosomes which arose from the cross of *T. turgidum* selection CI 13423 x *T. monococcum* var. *boeoticum* made by Mann and Lucken (1967). The 29-chromosome plant used to identify the *T. monococcum* chromosome was produced from seed obtained from Dr. S.S. Maan, Department of Agronomy, North Dakota University, Fargo, North Dakota, U.S.A. by Dr. B. Friebe, Department of Plant Pathology, Kansas State University, Manhattan, Kansas, U.S.A. The requisite cytological analysis of this plant was conducted by Dr. Friebe and the results made available for this investigation.

B. Ribosomal DNA clones

The 5S rRNA gene clone, pSCT7 (Scoles *et al.* 1987), carrying an ampicillin resistance gene, was obtained from Dr. G.J. Scoles, Department of Crop Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. pSCT7 carries the plasmid pUC8, in which a 500 bp sequence of the 5S rDNA gene from rye was inserted into the Bam H1 site. The 26S rDNA gene clone, pTA80 (unpublished), carrying a tetracycline resistance gene, was obtained from Dr. J. Dvořák, Department of Agronomy and Range Science, University of California, Davis, CA, U.S.A. pTA80 carries the plasmid pBR322, in which a 600 bp sequence of the nontranscribed spacer region between the 18S+26S repeating units in the rDNA region from *T. aestivum* (hereafter referred to as 26S rDNA) was inserted at the EcoR1 and BamH1 sites.

II. Methods

A. Production of autotetraploids

Autotetraploids were induced in the true-breeding diploid line 89 of *T. monococcum* by Dr. J. Kuspira. Approximately 200 seeds of the diploid accession were germinated and immediately treated with 0.1% colchicine for 6 hours. The germinated seeds were subsequently washed in water and planted in earthenware pots containing a mixture of loam soil, peat, vermiculite, and sand in a 2:1:1:1 ratio. The plants were grown in growth chambers in the Biological Science Building, University of Alberta, under the following condtions to ensure uniform and field-like conditions for growth: (i) 15 hours of 1400 ft-ca (1ft-ca=10.764391 lux) of cool white fluorescent light, 3 hours of 1600 ft-ca of incandescent and fluorescent light, followed by 6 hours of darkness; (ii) temperature of 21°C for the first 16 hours and 15°C for the rest of the day; and (iii) relative humidity of approximately 79-80% throughout the study.

Autotetraploid plants were identified at maturity on the basis of their heads, kernels, and leaves being much larger and their culms significantly thicker than those of the diploid progenitor. Tetraploidy was confirmed by determining the number of chromosomes in the somatic cells and microsporocytes of these plants.

B. Production of autotriploids

Seeds from plants identified as autotetraploids and from the true-breeding diploid lines 68, 89, 253, and 278 were sown 6.4 cm deep in moist soil of uniform fertility at the Genetics Field Laboratory at Ellerslie, Alberta. Crosses were made reciprocally by Dr. J. Kuspira between autotetraploids and line 89 in 1983. 2010 paper-thin, shrivelled seeds were obtained, all of which failed to germinate. Dr. J. Kuspira therefore repeated the crosses in 1984 and 1985 making them unidirectionally between autotetraploids as female parents and lines 68, 253, and 278 as male parents. Mitigating circumstances prevented the use of line 89 in crosses in both 1984 and 1985. The production of autotriploids was ensured by excising and culturing the embryos from the seeds of these latter crosses.

C. Culturing of embryos

Fifteen to sixteen days after crossing, florets from successful $4n$♀ x $2n$♂ pollinations were collected and rinsed in a bleach solution (2% Clorex) for one minute. Their embryos were excised under aseptic conditions and placed within sterilized glass tubes, each containing 25ml of a modified B5 medium devised by Gamborg *et al.* (1968) and modified by Kao and Kasha (1969). The tubes were incubated in the dark at 21°C until germination and root formation in the embryos were evident (approx. 3 to 5 days). They were then transferred to growth chambers with an 18 hour photoperiod and maintained there until the 2-leaf stage (Fig. 4). These seedlings were then potted in a soil mixture and placed in growth chambers under conditions previously described.

D. Production of trisomics

The protocol for the generation of trisomics is outlined in Fig. 5. For this investigation, the majority of the heads on the autotriploids that were produced by Dr. J. Kuspira in both 1984 and 1985 were allowed to self-fertilize. A minority of the heads were used in crosses with the diploid line 68 in 1984. Some of them were used only as female parents in crosses with lines 68 and 89 in 1985.

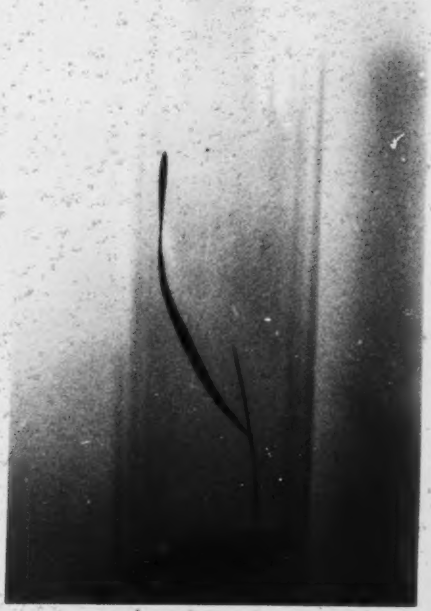


Fig. 4. A 15-days old *in vitro* cutured *T. monococcum* triploid seedling derived from an excised 16-day old embryo from the cross autotetraploid♀ x diploid♂.

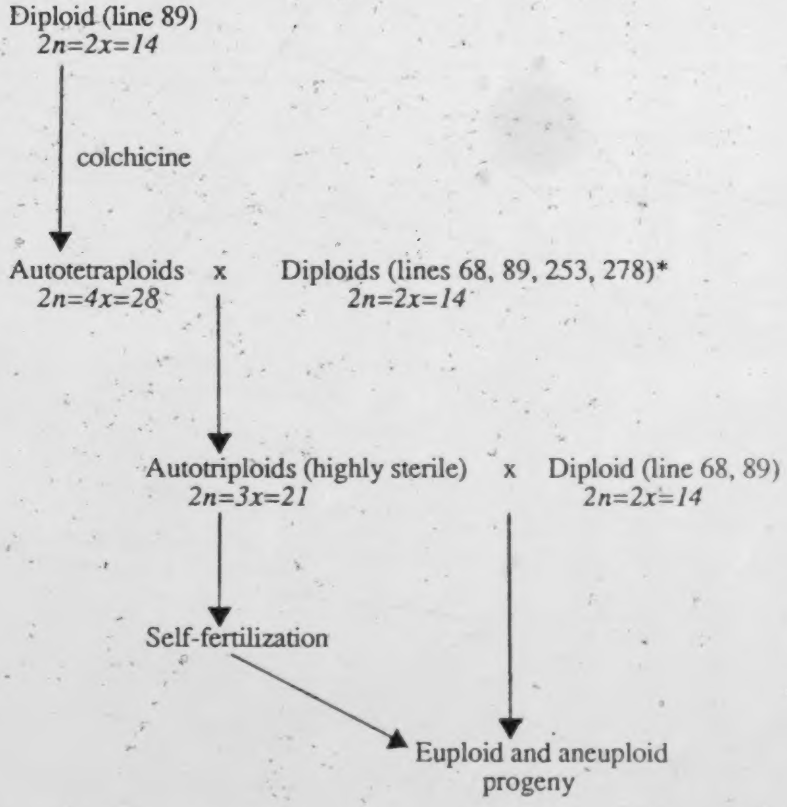

Diploid (line 89)
$2n=2x=14$
colchicine
Autotetraploids
$2n=4x=28$
x
Diploids (lines 68, 89, 253, 278)*
$2n=2x=14$
Autotriploids (highly sterile)
$2n=3x=21$
x
Diploid (line 68, 89)
$2n=2x=14$
Self-fertilization
Euploid and aneuploid progeny

Fig. 5. Protocol for production of primary trisomics in *Triticum monococcum*.
* Line 89 was used as the male parent in 1983 and the other three lines were used as the male parent in 1984.

E. Standard cytological analyses

1. Mitosis

Analysis of the chromosome complements of somatic cells was carried out on root-tip cells from seedlings or young plants. The root-tips were pretreated in cold water (4°C) for 24 hours to arrest mitotic metaphase chromosomes and fixed them in Carnoy's solution I (3:1 ethanol - acetic acid) (Carnoy 1886) and kept at 4°C for 1-2 weeks. The root-tips were then hydrolyzed in 1N HCl at 60°C for 10 minutes and stained with Feulgen (1% solution) prepared according to Sharma and Sharma (1965). Meristematic tissue of the root-tips that stained dark purple after 30 minutes to 1 hour was washed in distilled water. This darkly stained tissue was squashed on a slide in a drop of 45% acetic acid, tapped with a glass rod and covered with a coverslip. The slide was then pressed firmly to facilitate chromosome spreading. The cytological preparations were observed with a Wild phase contrast photomicroscope (Model 2476) under bright field. Slides containing cells with good chromosome spreads at metaphase and anaphase were selected for study and made permanent using a modification of the quick-freezing method (Conger and Fairchild 1953).

The relative length of each chromosome was determined as follows:

$$\frac{\text{Length of chromosome}}{\text{Total length of all chromosomes in the genome}}$$

2. Meiosis

Chromosome behaviour during meiosis was observed in microsporocytes obtained from spikes collected at the appropriate preheading stage. These spikelets were fixed in Carnoy's solution II (6:3:1 ethanol-chloroform-acetic acid) for approximately 1 week, and then transferred to 70% ethanol. Single anthers were immersed in a drop of 1% aceto-carmine solution on a glass slide. The aceto-carmine solution was prepared according to Sharma and Sharma (1965). Subsequently, the anthers were cut, macerated, the debris removed and the solution on the slide containing the meiocytes was covered with a coverslip. The slides were then gently heated and pressed firmly to facilitate chromosome spreading. Slides containing cells with good chromosome spreads at D, MI and AI were selected for study. These slides were made permanent using the method alluded to previously and subsequently examined under a microscope at 500X magnification. The cytological preparations were observed with the same microscope used for studying chromsome complements in somatic cells.

F. C-banding

The Giemsa C-banding technique, described by Giraldez *et al*. (1979), was modified and used to identify the chromosomes. Initially root-tips were pretreated and fixed in a manner similar to that for the standard analysis of somatic chromosome complements. They were transferred to 45% acetic acid for a few minutes to soften the meristematic tissue. Cells were sqeezed out from the tissue in a drop of 45% acetic acid on a glass slide. After placing a coverslip on the solution, the slide was heated and pressed to facilitate the spreading of chromosomes in the cells. The coverslips were removed using the quick freezing method and the slides were dipped in 98% ethanol overnight to dehydrate the tissues. The slides were then air dried and stained with Giemsa (Fisher Sci. Co.) using the following procedure: The cells on the slides were treated in 0.2N HCl at 60°C for 2.5 minutes and washed in distilled water. They were then dipped in a saturated $Ba(OH)_2$ solution at room temperature for 7 minutes and again washed in distilled water. The slides were subsequently incubated in 2 X SSC solution at 60°C for 1 hour and once again washed in distilled water. Finally, they were stained in 3% Giemsa in 0.15M phosphate buffer (pH 7) for 10 minutes. The intensity of the staining was checked every two minutes until the desired staining was obtained. Upon obtaining optimum C-bands, the slides were briefly rinsed in distilled water, blotted on filter paper and air dried. The slides were then mounted with Permount (Fisher Sci. Co.).

Chromosome measurements were carried out on 15 complete and well spread mitotic metaphases of diploid line 89 of *T. monococcum* to establish a karyotype of the C-banding patterns of its chromosomes.

G. Staining of nucleoli

Nucleoli were detected using the silver staining technique (Ag-NOR) described by Lacadena *et al.* (1984) with slight modifications. Root-tip pretreatment, fixing and squashing of cells were similar to that described for C-banding. The remainder of the protocol was as follows: Several hours after removing the cover slips, the slides were immersed in Carnoy's solution I and flame dried. Then one or two drops of freshly prepared silver staining solution was added to each slide and coverslips were placed on the solution. The silver staining solution was made by dissolving 1g of $AgNO_3$ in 1 ml of a solution of 0.02g of sodium citrate ($C_6H_5Na_3O_7$-$2H_2O$) in 500 ml of distilled water adjusted to pH 3 with formic acid. The slides were then placed in a moist chamber at 55 - 60^{o}C for 1 hour. The intensity of the reaction was monitored under a microscope every 5 minutes until optimum staining was obtained. The slides were then counter stained in a 3% Giemsa solution for 5 minutes. The nucleoli were detected by observing interphase cells for silver stained regions.

H. *In situ* hybridization

1. Chromosome preparation

Root-tips were pretreated and fixed in a similar fashion to that described for standard karyotypic analysis. The root-tips were then washed in 70% ethanol, followed by 30% ethanol, and rinsed twice in distilled water for a duration of 3 minutes in each solution. Cells on the slides were squashed as described for C-banding except that they were not heated. After removing the coverslips using the N$_2$ freezing method, the slides containing several cells with well spread chromosomes were kept for 1 - 2 months in a descicator at room temperature until required for use.

For meiotic chromosome preparations, immature spikelets were fixed in Carnoy's solution I at room temperature over night. The spikelets were then transferred to 70% ethanol and maintained at 4^{o}C until required for use. The anthers were screened for the MI or AI stages of meiosis and then squashed in a drop of 45% acetic acid. The slides containing meiocytes with well spread chromosomes were kept in a descicator at room temperature until required for use.

2. Plasmid DNA extraction

The procedure used in isolating plasmid DNA from transformed *E. coli* strains containing the appropriate pSCT7 and pTA80 was that outlined by Maniatis *et al.* (1982). A single bacterial colony inoculated in 10 ml of liquid LB medium (10g Bacto trypton, 5g yeast extract, and 10g NaCl in 1 liter of water) containing the appropriate antibiotics was incubated at 37^{o}C overnight under vigorous shaking conditions. A volume of 5ml of this overnight culture was mixed with 500 ml of LB medium and incubated in a shaker at 37^{o}C for 10 - 12 hours. The bacterial cells were then precipitated at 4000 r.p.m. for 10 minutes at 4^{o}C. The pellet was resuspended in a 10 ml of 0.5 mM Tris buffer (pH8.0) and 10% sucrose solution. 2 ml of freshly prepared lysozme (50 mg/ml) and 8 ml of 0.25 M EDTA were added to the suspension which was then placed in ice for 10 minutes. 4 ml of 10% SDS were then added followed by gentle shaking and addition of 6 ml of 5 M NaCl. The culture was placed in ice for 1 hour with gentle shaking every 10 minutes. The lysed cells were transferred to a Beckman SW28 centrifuge tube and cetrifuged at 27000 r.p.m. for 1 hour at 4^{o}C. The supernatant was collected and an equal volume of cold isopropanol (-20°C) added to it. The lysate was precipitated at 4500 r.p.m. for 10 minutes at 4°C. The pellet was dissolved in 30 ml of TE buffer (10 mM Tris ph8, 1 mM EDTA). Cellular proteins from the lysate were removed with phenol, phenol and chloroform, and chloroform, sequentially. The DNA was precipitated with cold 95% ethanol (-20°C) and centrifuged at 4500 r.p.m. for 5 minutes at 4°C. The DNA pellet was completely dissolved in 9 ml of TE buffer and kept at 4°C for 2 - 3 days. The DNA suspension was then mixed with an equal volume of CsCl (1 mg/ml dH_2O). A volume of 0.8 ml of ethidium bromide (10 mg/ml dH_2O) per 10 ml of CsCl solution was added to the DNA-CsCl mixture. The mixture was transferred to a Beckman Quick-Seal centrifuge tube (13x51 mm) and centrifuged at 48000 r.p.m. for 12 hours at 20°C. The tube containing DNA was observed under long wave length U.V. light to contain two bands. DNA from the bottom band, which represents plasmid DNA, was collected and recentrifuged in a Beckman Quick-Seal cetrifuge tube (13x51 mm) at 54000 r.p.m. for 6 hours at 20°C. A thick and distinct band of DNA was formed and collected. The ethidium bromide was removed with several washes of butanol. The CsCl was eliminated from the DNA solution by dialyzing in TE overnight. The concentration of the pure plasmid DNA was then measured by O.D. measurement in a spectrophotometer (LKB Biochrom, ULTROSPEC 4050).

3. Isolation of cloned fragments

DNA restrictions were carried out as suggested by Maniatis *et al.* (1982). 102 *u*l (132 *u*g of DNA) of the pSCT7 DNA solution was digested with 180 units of BamH1 using the supplier's (BRL) appropriate buffer. 150 *u*l (45 *u*g of DNA) of the pTA 80 DNA solution was digested with 80 units of HindIII using the supplier's (BRL) appropriate buffer and then digested with 80 units of EcoR1 (BRL) in the appropriate salt concentration in the buffer. The restriction cuts were confirmed in 0.8% agarose gel. Upon restriction, the specific-DNA fragment was separated from the plasmid DNA fragment in 1% low melting point agarose (Sigma Co.) gel containing 3 *u*g of ethidium bromide per 100 ml of gel buffer. The gel was illuminated with long wave length U.V. light and the band containing the rDNA identified and cut out of the gel. The gel slice was placed in a 15 ml sterilized plastic tube and melted completely in a 70°C water bath. Then 4 volumes of 0.25 M NaCl in TE buffer were added to the molten gel , mixed well, and incubated at 70°C for 10 minutes. Subsequently, the tubes were transferred to a 4°C water bath for 10 minutes. The diluted gel solution was loaded on a PRE PAC^{Tm} column (BRL) which was previously equilibrated with 5 ml of equilibrium buffer (0.25 M NaCl in TE pH 7.2). After washing off the agarose and gel impurities from the column with 5 ml of equilibrated buffer, the DNA was eluted from the column with 0.1 ml of elution buffer (1 M NaCl in TE), three times. The effluent was pooled and the fragment DNA was precipitated with 0.6 ml of cold 95% ethanol (-20°C). The precipitate was placed in a freezer (-70°C) for 10 minutes and then centrifuged for 15 minutes at 1200 r.p.m.. The nucleic acid pellet was washed carefully with cold 80% ethanol (-20°C) and dried under vaccum for 5 minutes. The purified DNA was subsequently dissolved in 50 *u*l of TE.

4. Oligolabelling

Oligolabelling reactions were set up according to the supplier's (Pharmacia) manual. Each isolated rDNA fragment (1 *u*g) was denatured at 90°C for 15 minutes and transferred to ice directly. The reaction mixture of 34 *u*l of dH$_2$O (distilled water), 2 *u*l of BSA, 80 *u*Ci of [^{3}H]dCTP (DuPont) and 2 *u*l of Klenow fragment was incubated for 2 hours at room temperature. The reaction was terminated with 20 *u*l of stop buffer and 180 *u*l of dH$_2$O. The unincorporated [^{3}H]dCTP was removed according to Maniatis *et al.* (1982) using a Sephadex G50 DNA grade column. The specific activity for the pSCT7 and pTA80 probe was determined to be 4.7x10^7 c.p.m. and 3.9x10^7 c.p.m., respectively.

5. *In situ* hybridization

The procedure utilized for *in situ* hybridization was basically similar to that of Dvořák *et al.* (1984). Each slide that was prepared for *in situ* hybridization was treated with 20 *u*l of RNase mixture (20 *u*l of RNase of 100 ug/1ml d H_2O, 880 *u*l of dH_2O, and 100 *u*l of 2X SSC) for 30 minutes at room temperature. The coverslips were removed in dH_2O and the slides were washed for 3 minutes in each of dH_2O, 70% ethanol, and 95% ethanol. The slides were dried under a hair-drier for 10 minutes. The chromosomal DNA was denatured and neutralized by immersing the slides in a saturated $Ba(OH)_2$ solution for 6 minutes and 1 N HCl for 30 seconds, respectively, at room temperature. This was followed by sequential washes in dH_2O, 70% ethanol, and 95% ethanol for 3 minutes each at room temperature. The slides were dried under a hair-drier again. Then, 15*u*l (1 ng of probe DNA) of hybridizing mixture, containing 50% formamide, 3 X SSC, 10% dextran sulfate, 15 *u*g of sonicated and denatured Lambda DNA, and 15 ng of denatured probe DNA, was applied to each slide. A 22x22 mm coverslip was placed on each slide and sealed with rubber cement. The slides were heated (65^{o} - 70^{o}C) for 30 seconds to denature all the chromosomal DNA and subsequently incubated overnight in a water bath at 40^{o}C. The following morning, the rubber cement was peeled off and the coverslips removed by dipping the slides in 3X SSC with 50% deionized formamide at 45^{o}C for 5 minutes. The slides were then washed sequentially with 2X SSC in 0.1% SDS for 10 minutes three times at 45^{o}C, three times at room temperature with 1X SSC for 10 minutes, and for 5 minutes with 70% ethanol and 95% ethanol, respectively, at room temperature. Finally, the slides were dried under a hair-drier.

6. Autoradiography of the *in situ* preparations

The dried slides were dipped in a 50% Kodak NTB emulsion solution under red safety light. After drying for 1 hour in the safety light, the slides were placed in a black slide box and refrigerated at 4°C for 2 weeks. The slides were then developed under the red safety light at room temperature. They were placed in dH_2O for 1 minute and transferred sequentially through 50% Dektol developer (Kodak) for 2 minutes, dH_2O for 10 seconds, Kodak fixer for 5 minutes, dH_2O for 5 minutes, and running water for 10 minutes. The slides were then stained with 10% eosinate (Sigma Co.) solution.

I. Determination of plant morphological phenotypes

All the morphological studies of diploids, autotriploids, autotetraploids and trisomics were carried out in 1986 and 1987 on plants grown at the Genetics Field Laboratory under conditions described previously. Measurements of all characters were made on the two main culms of plants at each ploidy level and of trisomic plants in each trisomic line. The measurements in each of the two years were almost identical and therefore the values given in Table10 represent the ranges and means for this two year period. Heading date values given in Table10 represent number of days to heading from date of seeding. The units of measurement for all characters, except the number of spikelets per head, are given in brackets (Table 10).

J. Photography

Photographs of spikes, seeds and leaves were taken using a Pentax 35mm camera. Photomicrographs of meiotic cells at MI and AI, somatic cells at metaphase, and *in situ* hybridizations were prepared using a Zeiss Photomicroscope (Model 64447) and panchromatic ASA 32 film. Photomicrographs of C-banded chromosomes at metaphase were taken with the same photomicroscope using either Imagecapture AHU microfilm or Kodak Technical Pan 2415 black and white film. The photographs of spikes, seeds, leaves, and aceto-carmine stained meiotic chromsomes were printed on Kodabromide F3, black and white paper. The photographs of somatic chromosomes stained in a standard manner, as well as C-banded and *in situ* hybridized chromosomes were printed on Kodabromide F5, black and white paper.

K. Determination of breeding behaviour and fertility

The breeding behaviour of trisomics was determined by self-fertilizing the aneuploids as well as crossing them reciprocally with diploids. All crosses were carried out at the Genetics Field Laboratory. Percent fertility (percent seed set) was determined as the proportion of florets per spike that contained seed.

RESULTS and DISCUSSION

I. Breeding behaviour of autotriploids and production of trisomics

A. Breeding behaviour of autotriploids

The results of the self-fertilization of autotriploids as well as their reciprocal crosses with diploid line 68 are shown in Table 3. Analysis of meiotic chromosome behaviour of autotriploids showed that they produced a complete range of meiotic products from *n* through all possible unbalanced types to *2n* (Kuspira *et al.* 1986b). Therefore, one should expect all possible euploid and aneuploid types of progeny to be generated from self-fertilizing autotriploids and crossing them with diploids. This expectation, however, was not realized. Autotetraploid progeny were produced only from self-fertilizations of autotriploids. Disregarding *2n*♀ x *3n*♂ crosses, autotriploids produced approximately the same proportions of diploid and aneuploid progenies in both *3n*♀ x *2n*♂ crosses and self-fertilization of autotriploids (Table 3) with diploids, single, double, and triple trisomics being present in approximate average percentages of 54.0, 42.3 1.4, and 1.0, respectively. The single trisomics were much more numerous than the double and triple ones because they are genetically the least unbalanced. Although all the expected euploid types were produced, only a fraction of all the possible aneuploid progeny types were obtained. This observation has also been made in many other diploid plant species, e.g. perennial ryegrass (Myers 1944), tomato (Rick and Barton 1954), spinach (Janick *et al.* 1959), pearl millet (Gill *et al.* 1970), and rice (Khush *et al.* 1984).

Only 3 of the 6 possible trisomic types of progeny were produced from self-fertilization of autotriploids and reciprocal *3n* x *2n* crosses. These were the single ($2n+1$), double ($2n+2$) and triple ($2n+3$) trisomics. The results of crosses between *2n*♀ x *3n*♂ indicate that only balanced male gametophytes gave rise to viable and functional gametes which then effected fertilization and produced viable *2n* and *3n*

Table 3. Number of euploid and aneuploid types of progeny produced by autotriploid *T. monococcum*.

Year	Parents	Seeds	No. of seeds that did not germinate	Types of progeny (numbers and percent of each)									
				2n	*2n+1*	*2n+2*	*2n+3*	*2n+4*	*2n+5*	*2n+6*	*3n*	*4n*	Total
1984	*2n*♀ x *3n*♂	62	4	55(94.8%)	0	0	0	0	0	0	3(5.2%)	0	58
	3n♀ x *2n*♂	44	10	18(52.9)	14(41)	1(2.9)	0	0	0	0	1(2.9)	0	34
	3n(S.F.*)	34	8	14(53.8)	11(42.3)	0	1(3.8)	0	0	0	0	0	26
1985	*3n*♀ x *2n*♂	139	37	56(54.9)	45(44.1)	1(0.9)	0	0	0	0	0	0	102
	3n(S.F.)	111	54	30(52.6)	24(42.1)	1(1.7)	0	0	0	0	0	2(3.5)	57
	Total	390	113	173	94	3	1	0	0	0	4	2	277

* S.F. = Self-fertilized.

progenies. In all likelihood male gametophytes with unbalanced chromosome constitutions aborted. Those that remained viable and functional, either did not effect fertilization because of certation or gave rise to aneuploid zygotes or embryos that subsequently aborted. These results further indicate that only balanced and unbalanced *n+1*, *n+2*, and *n+3* functional gametes are produced on the female side in these polyploids. Absence of the other multiple trisomic types was most likely due to the abortion of *n+4*, *n+5*, and *n+6* female gametophytes or to the subsequent abortion of the zygotes or embryos with multiple aneuploid chromosome constitutions.

Plant species can be broadly classified into two groups with respect to the range of aneuploids that can be recovered from autotriploids derived from the diploid progenitor (Table 4). The first group is highly intolerant of aneuploidy. It consists of such species as spinach (Janick *et al.* 1959), barley (Tsuchiya 1960), tomato (Rick 1971), rice (Khush *et al.* 1984) and *T. monococcum* (results of this thesis, see Table 3). The intolerance of extra chromosomes in these species is always associated with a lack of vigor, mutant phenotypic expression, and reduced fertility. The extent of deviation from the normal phenotype depends on the degree of aneuploidy. Each specific chromosome in single trisomics produces a specific syndrome of effects. Therefore each of these aneuploids and multiple trisomics are usually distinguishable from each other and from their diploid progenitors.

The second group of species is highly tolerant of aneuploidy. It includes species such as *Z. mays* (McClintock 1929, Punyasingh 1947), *C. unguiculata* (Vasek 1956), *Solanum* spp. (Vogt and Rowe 1968), and *Petunia hybrida* (Rick 1971). A complete range of viable trisomics has been obtained in *P. hybrida* (Table 4). Most of the possible trisomic types have been observed in other species that belong to this group (Table 4). Moreover, the different trisomics in each species within this group are morphologically indistinguishable from each other and

Table 4. Range (%) of progeny from 3n♀ x 2n♂ crosses in different plant species.

Species	Chromosome no.													Source
	2n	*2n+1*	*2n+2*	*2n+3*	*2n+4*	*2n+5*	*2n+6*	*2n+7*	*2n+8*	*2n+9*	*2n+10*	*2n+11*	*2n+12*	
Lotus pedunculatus (*n*=6)	62.7	35.8	1.5	0	0	0	0=3*n*							Chen and Grant 1968
Triticum monococcum (*n*=7)	54.0	42.3	1.4	1.0	0	0	0	0=3*n*						This thesis
Pennisetum typhoides (*n*=7)	64.8	34.1	0	0	0	0	0	1=3*n*						Gill *et al.* 1970
Hordeum spontaneum (*n*=7)	30.0	53.3	10.0	3.3	0	0	0	0=3*n**						Tsuchiya 1960
Lycopersicum esculentum (*n*=12)	38	44	16	1	0	0	0	0	0	0	0	0	1=3*n*	Rick and Barton 1954 Rick 1971
Oryza sativa (*n*=12)	2.8	27.8	34.7	11.1	4.2	0	0	0	0	0	0	0	0=3*n*	Khush *et al.* 1984
Lolium perenne (*n*=7)	25.2	43.7	22.7	5.0	3.4	0	0	0=3*n*						Myers 1944
Petunia hybrida (*n*=7)	9	32	35	14	3	5	2	0=3*n*						Rick 1971
Medicago spp. (*n*=8)	39.8	41.9	11.8	3.2	0	0	0	0	0=3*n***					Kasha and McLennan 1967
Clarkia unguiculata (*n*=9)	18.6	16.3	30.2	11.6	7.0	7.0	2.3	7.0	0	0=3*n*				Vasek 1956
Zea mays (*n*=10)	2	12	29	25	19	9	3	1	0	0	0=3*n*			McClintock 1929 Punyasingh 1947
Solanum-series *tuberosa* (*n*=12)	17.7	44.6	14.6	12.3	1.6	0.8	4.6	3.8	0	0	0	0	0=3*n*	Vogt and Rowe 1968

* 3.4% of the progeny were not identified cytologically.

** Two tetraploids and one hexaploid also occurred among the progeny.

from their diploid progenitors. Their viablity is not reduced or only to a small extent and they are highly fertile. For example, some are as fertile as the diploids in *C. unguiculata*. It has been demonstrated that genetic duplication is minimal in members of the first group but more extensive in species of the second group (Rhoades 1951; Rick 1971; Helentjaris *et al.* 1988; Wendel *et al.* 1989). It is therefore likely that the extent of genetic duplication determines the level of tolerance of aneuploidy in a species. Tolerance of more than one extra chromosome may also be dependent upon the interaction of genes. Rick (1971) has shown that there is less interaction of genes in plants of the second group than in the case of species in the first group. Tolerance of aneuploidy may also be contingent on the mode of fertilization of a species. Members of the highly intolerant groups are self-fertilizing whereas members of the highly tolerant ones are cross-fertilizing species.

A more complete understanding of tolerance vs. intolerance of aneuploidy in these species requires a detailed additional genetic information.

B. Production of trisomics

Table 3 shows that none of the progeny from the *2n*♀ x *3n*♂ cross conducted in 1984 were trisomics. Therefore, the cross was not repeated in 1985. Eighteen of the 78 seeds derived from the *3n*♀ x *2n*♂ cross as well as the self-fertilization of the autotriploids in 1984 did not germinate. The plants from the remaining seeds were assigned arbitrary numbers from 1 to 60. Cytological analysis of their microsporocytes revealed that one of the plants was an autotriploid, 32 were diploids, 25 were primary single trisomics, one was a double trisomic, and one was a triple trisomic (see Fig. 6). A trisomic line consisting of diploids and trisomics was generated from each of the primary trisomics by self-fertilization. Each of these lines was maintained by self-fertilization of the trisomics. Seven of these lines (26, 34, 39, 40, 47, 52, and 58) were lost because of low fertility and a failure to produce trisomics. The remaining 18 trisomic lines were maintained and

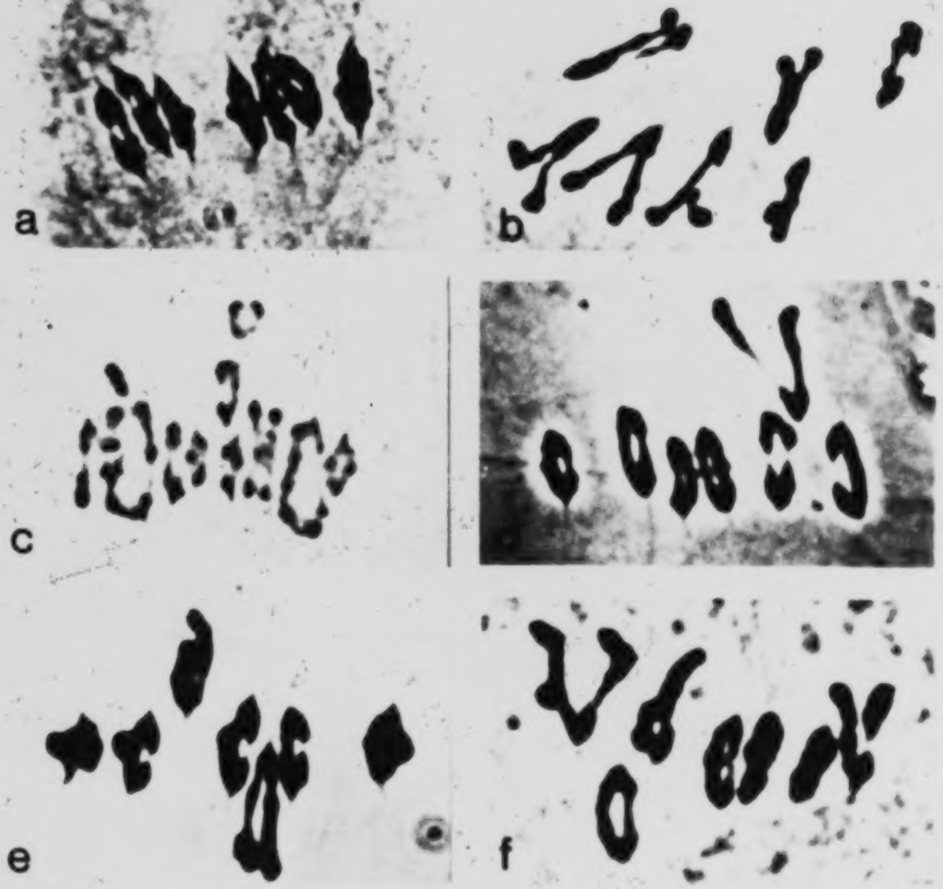


Fig. 6. Typical metaphase I pairing in *T. monococcum* euploids and trisomics. (a) Diploid. (b) Autotriploid. (c) Autotetraploid. (d) Single trisomic. (e) Double trisomic. (f) Triple trisomic. (Magnification : 1300 X)

studied cytologically, phenotypically, and with respect to their breeding behaviour and fertility. *The results presented in this thesis were derived from investigations conducted with these 18 trisomic lines.*

Of 250 seeds obtained from the crosses made in 1985, ninety-one failed to germinate. The remainder gave rise to progeny types given in Table 3.

II. Identification of chromosomes of *T. monococcum* and its primary trisomics

A. Standard cytological analysis

The sizes and shapes of Feulgen-stained chromosomes observed at metaphase in somatic cells of diploid line 89 and trisomics (Fig. 7) are similar to those observed by Coucoli and Skorda (1966), Giorgi and Bozzini (1969b) and Kerby and Kuspira (1988a) in other *T. monococcum* accessions. Depending on the accession line studied, a small percentage of cells showed either one or two pairs of chromosomes with very small satellites (SATs) at the termini of their short arms (Upadhya and Swaminathan 1963; Coucoli and Skorda 1966; Giorgi and Bozzini 1969b; Waines and Kimber 1973; Anastassova-Kriseva *et al.* 1978). However, chromosomes that had satellites were rarely observed in the cells of line 89. The consensus of various studies is that the chromosomes in the diploid complement of this species are very simlilar in gross morphology. Table 5 shows a comparison of the relative lengths and arm ratios of the chromosomes of *T. monococcum* with those of the A genome of *T. aestivum* (Gill 1987). The relative lengths and arm ratios were similar for chromosomes 1A, 2A, 3A, 6A and 7A of *T. aestivum* and their homologues in *T. monococcum*. The largest differences in these two parameters were observed for chromosomes 4A and 5A and their equivalents. In *T. monococcum*, the chromosomes in karyotypes from standard preparations were

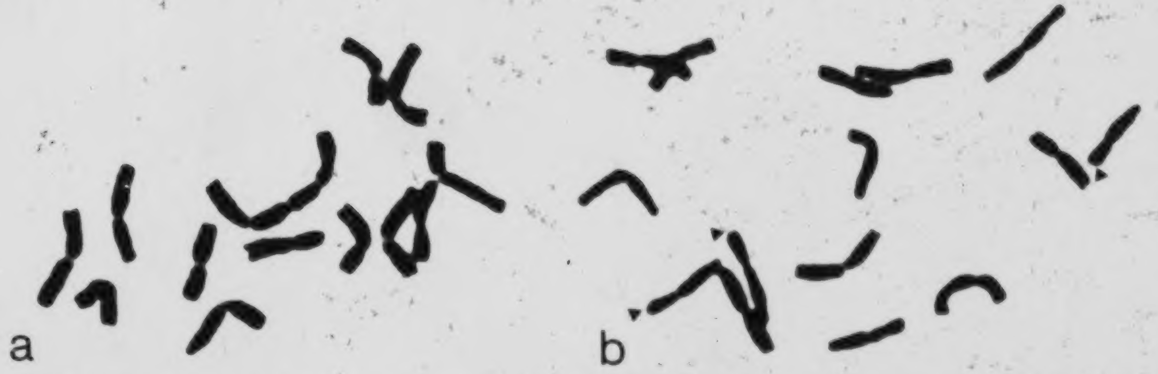


Fig. 7. Standard Feulgen stained mitotic metaphase chromosomes of (a) diploid line 89 ($2n = 14$) and (b) trisomic line 7 ($2n = 14 + 1$) of *T. monococcum*. Triangles point to minute satellites on three of the chromosomes. (Magnification : 1300 X)

Table 5. Relative lengths and arm ratios of somatic metaphase chromosomes of *T. monococcum* and the A genome chromosomes in *T. aestivum* .

Chromosome	*T. aestivum**			*T. monococcum*	
	Length (*um*)	Relative length	Arm ratio (L/S)	Relative length	Arm ratio (L/S)
1A	11.1	13.9	1.9	14.2	1.8
2A	12.5	15.7	1.3	16.1	1.3
3A	11.5	14.4	1.3	16.1	1.4
4A	11.9	14.9	1.7	12.7	1.2
5A	11.5	14.4	1.8	12.6	1.6
6A	9.8	12.3	1.1	12.6	1.1
7A	11.3	14.2	1.0	15.7	1.2

* The lengths and arm ratios were determined by Gill (1987).

morphologically similar and therefore could not be unequivocally identified. Consequently, identification of the chromosomes present in triplicate in the different trisomics could not be achieved with certainty using standard cytological procedures.

B. Identification using silver(Ag)-NOR staining, *in situ* hybridization and C-banding

1. Identification of chromosomes in diploids

a. Staining of nucleoli

A maximum of four nucleoli and therefore NORs were observed in diploid root-tip cells at interphase (Table 6). Thus two of the seven chromosome pairs in *T. monococcum* possess NORs and allow them to be distinguished from the others in the species. These results confirm the observations of Darvey and Driscoll (1972), Gerlach *et al.* (1980), Miller *et al.* (1980, 1983).

b. *In situ* hybridization

Hybridization with a 26S rDNA probe revealed that the rDNA hybridized to the terminal regions of the short arms of four chromosomes in the diploid somatic complement (Fig. 8, Table 6). The probe also hybridized to the short arms of the homologues of two chromosome pairs in meiocytes of the diploid (Fig. 9). These results support the conclusions from Ag-NOR staining, and those of Gerlach *et al.* (1980), Miller *et al.* (1983), that only four chromosomes (two chromosome pairs) in *T. monococcum* carry NORs. Moreover, these observations support the findings of Miller *et al.* (1980) and others who also showed that NORs are located next to the satellites of the SAT chromosomes in this species. The labelling pattern in one of these chromosome pairs was heavier than in the other (Figs. 8 and 15). Using a rDNA probe, Gerlach *et al.* (1980) and Miller *et al.* (1983) made similar observations. Gerlach *et al.* (1980) found that the probe hybridized lightly to the

Table 6. Maximum number of chromosomes in somatic cells of diploids and trisomics of *T. monococcum* that possess NORs as revealed by nucleolar staining and *in situ* hybridization.

Diploid and trisomic lines	Number of interphase nucleoli and NORs	Number of chromosomes showing *in situ* hybridization with		
		26S rDNA	5S rDNA	Mixture of 26S and 5S rDNA
Diploid	4	4	4	4
3	4	4	4	4
4	No data	4	4	4
6	No data	4	4	4
7	5	5	5	5
8	4	4	4	4
10	4	4	4	4
16	4	4	4	4
20	No data	5	5	5
22	4	4	4	4
28	5	5	5	5
29	4	4	4	4
33	No data	4	4	4
36	5	5	5	5
38	5	5	5	5
41*	5	No data	No data	No data
42	No data	4	4	4
50	5	5	5	5
51	5	5	5	5
57	4	4	4	4

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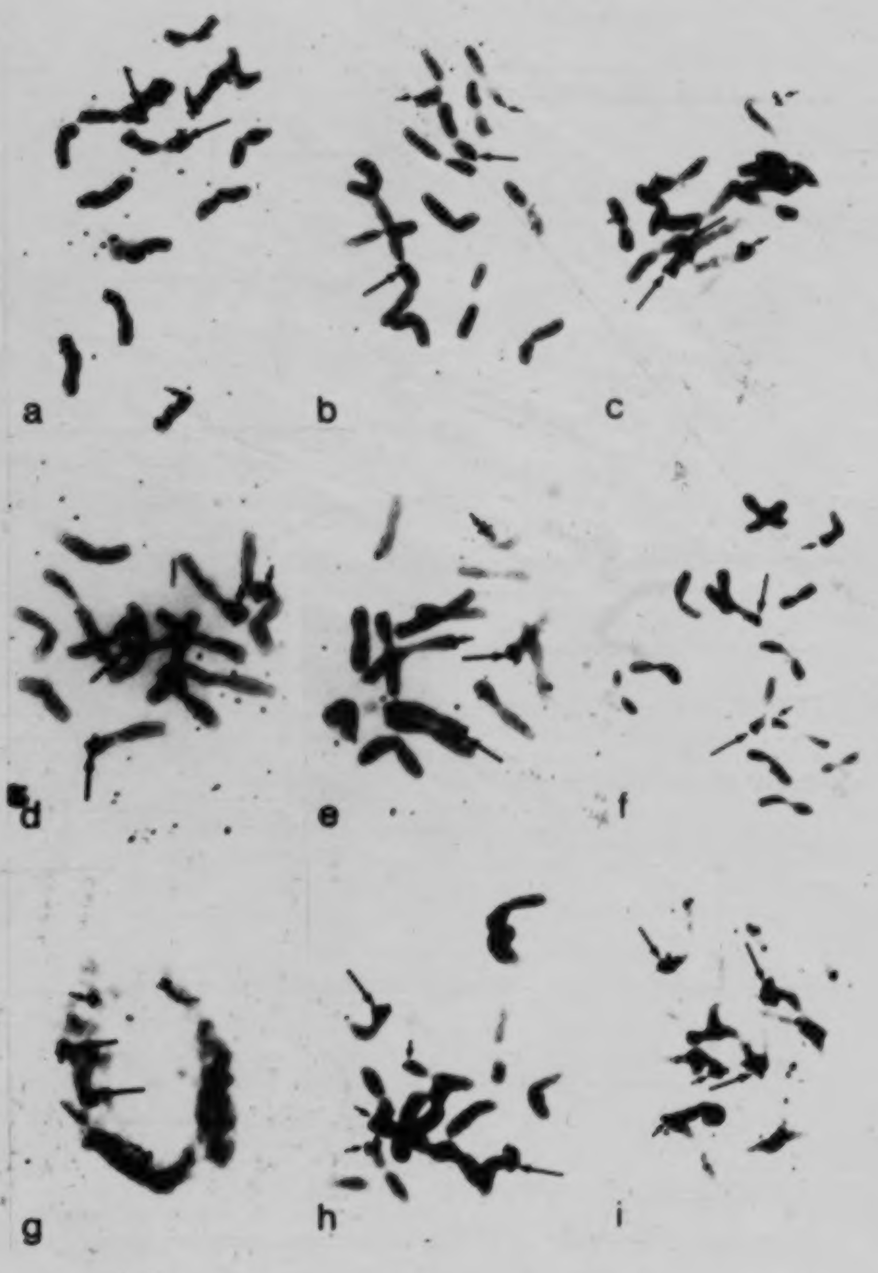
* The trisomic plant 41 arose in trisomic line 42 in which all other trisomics possessed chromosome 6A in triplicate.

Fig. 8. *In situ* hybridization of chromosomes in diploids and trisomics of *T. monococcum* at mitotic metaphase and interphase with 5S rDNA, 26S rDNA and mixture of the two probes. (a) Hybridization with 5S rDNA in diploid line 89. (b) Hybridization with 26S rDNA in diploid line 89. (c) Hybridization with mixture of both probes in diploid line 89. (d) Hybridization with 5S rDNA in a trisomic for chromosome 2A. (e) Hybridization with 26S rDNA in a trisomic for chromosome 6A. (f) Hybridization in a trisomic for chromosome 6A with both probes. (g) Hybridization with 5S rDNA in a trisomic from line 50, trisomic for SAT chromosome 1A or 5A. (h) Hybridization with 5S rDNA in a trisomic from line 50, trisomic for SAT chromosome 1A or 5A. (i) Hybridization with both probes in a trisomic from line 28, trisomic for SAT chromosome 1A or 5A.

Large arrows indicate heavy hybridization and small arrows indicate light hybridization. (Magnification : 1000 X)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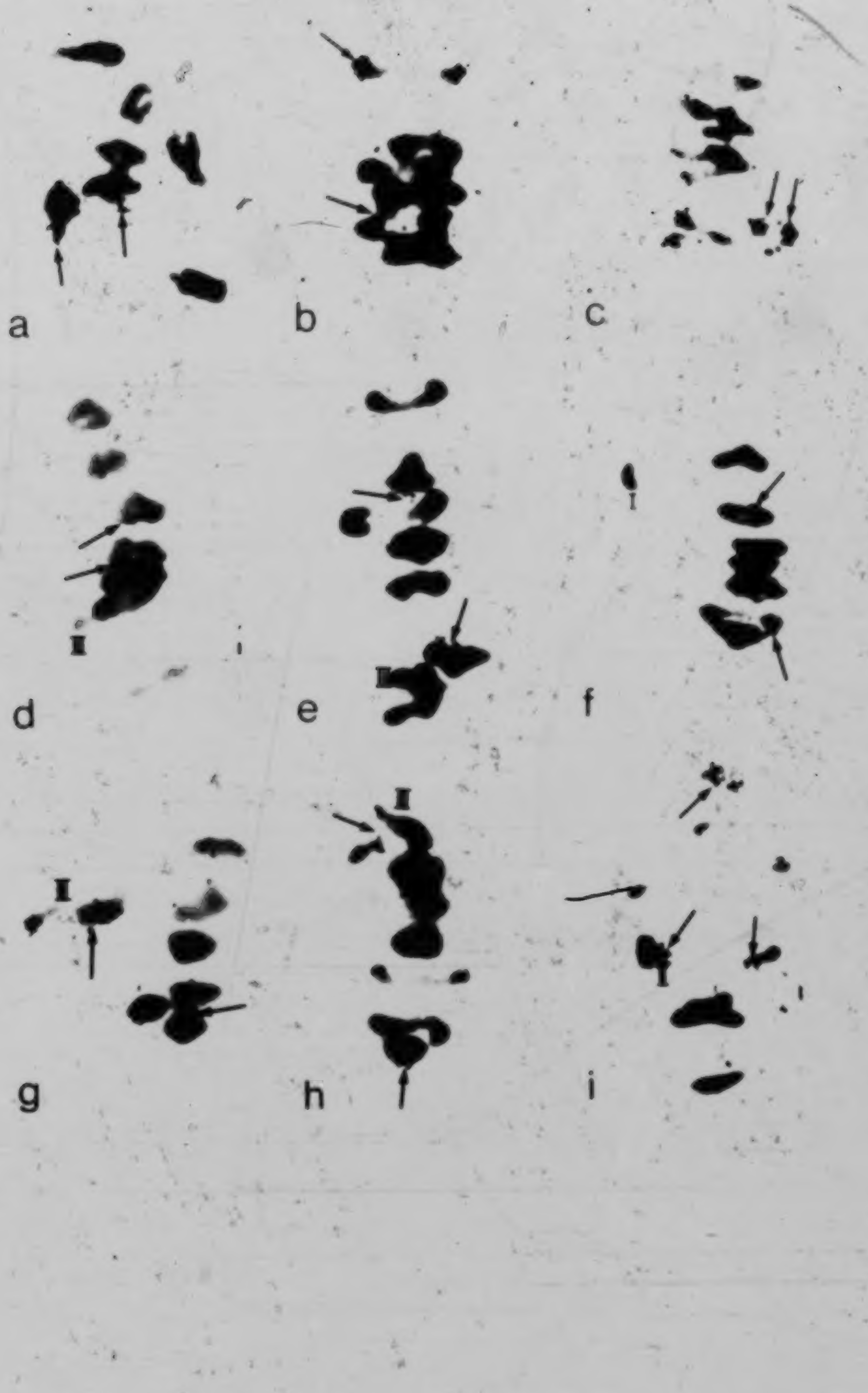
b
c
d
e
f
g
h
i

Fig. 9. *In situ* hybridization of chromosomes in microsporocytes of *T. monococcum* at D and MI with 5S rDNA, 26S rDNA and a mixture of the two probes. (a) Hybridization with 5S rDNA in diploid line 89. (b) Hybridization with 26S rDNA in diploid line 89. (c) Hybridization in diploid line 89 with a mixture of the two probes. (d) Hybridization in a trisomic from line 57 (trisomic for chromosome 6A). (e) Hybridization with 26S rDNA in a trisomic from line 22 (trisomic for chromosome 7A). (f) Hybridization with a mixture of the two probes in a trisomic from line 22 (trisomic for chromosome 7A). (g) Hybridization with 5S rDNA in a trisomic from line 28 (trisomic for one of the NOR-carrying chromosomes). (h) Hybridization with 26S rDNA in a trisomic from line 20 (trisomic for one of the NOR-carring chromosomes). (i) Hybridization with a mixture of the two probes in a trisomic from line 28 (trisomic for one of the NOR-carrying chromosomes).

Arrows points to Is, IIs, and IIIs which show hybridization with probes.

(Magnification : 1000 X)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1A pair of chromosomes whereas Miller *et al.* (1983) failed to detect any hybridization signals on this chromosome pair. Both groups of workers detected heavy labelling with the probe on both 5A chromosomes. Therefore, of the four SAT chromosomes, the two that hybridized lightly with the rDNA probe are in all likelihood chromosome 1A and the two that hybridized heavily are highly likely chromosome 5A. According to Szabo *et al.* (1977) and Appels *et al.* (1980), the extent of *in situ* hybridization in a given NOR of a chromosome is proportional to the number of rRNA genes in that region. The results of this investigation, as well as those of Gerlach *et al.* (1980) and Miller *et al.* (1983), imply that the NORs on chromosome 1A and 5A of *T. monococcum* carry low and high copy numbers, respectively, of the NOR genes.

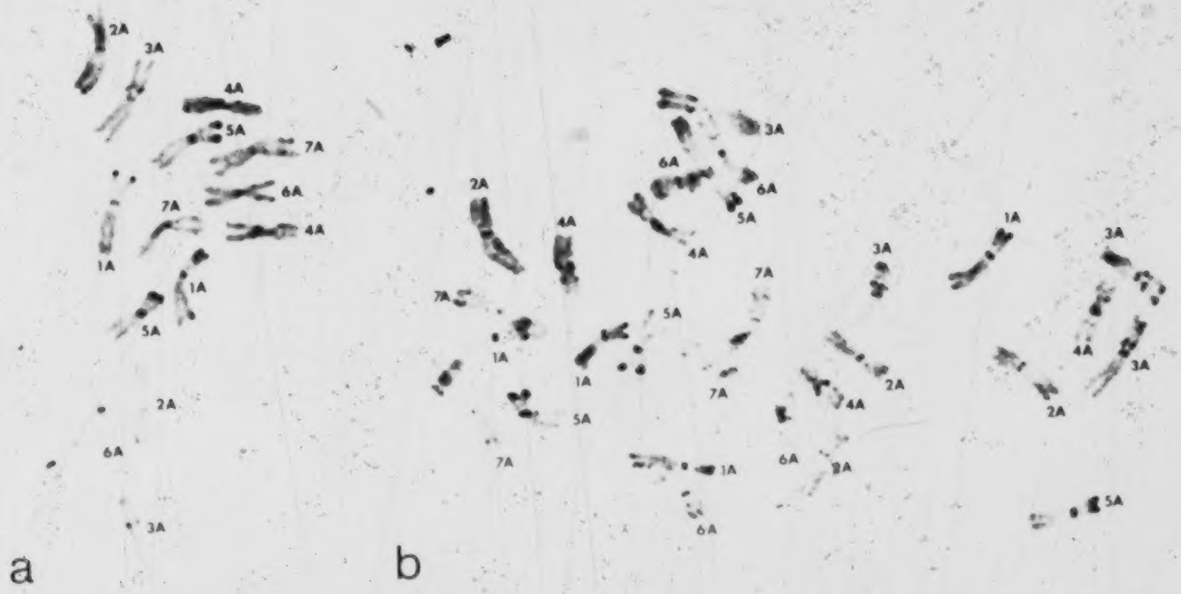
c. C-banding

i) General

Of the various banding methods used in identifying chromosomes, only the C- and N-banding techniques have proven to be useful in plants (Linde-Laursen 1975; Gerlach and Peacock 1980; Loidl 1983). In *T. aestivum*, all 21 chromosomes can be identified on the basis of their C-banding patterns (Endo 1986; Gill 1987); 16 of them can be identified using the N-banding procedure (Endo and Gill 1984a). C-banding has also been used to identify all the chromosomes in *T. turgidum* (Simeone *et al.* 1988). Presently, C-banding is the only banding method that can be used to identify chromosomes of *T. monococcum*. The original C-banding protocols used in this species by Gill and Kimber (1974a) and Kuz'menko *et al.* (1987) produced few and faint bands precluding identification of the chromosomes. Giraldez *et al.* (1979) modified the original C-banding methods and B. Friebe (Institut fur Pflanzenbau und Pflanzenzuechtung, Technische Universitat of Munchen, Munchen, Federal Republic of Germany) made additional changes (unpublished) in the technique Giraldez *et al.* of which revealed all the C-bands in *T. monococcum* chromosomes, and thus permiting their identification.

C-banded chromosomes in mitotic metaphases of the diploid (line 89) and the induced autotetraploid are shown in Fig. 10. A typical karyotype of the diploid is represented in Fig. 11. Characteristic C-bands were present in the region of the centromere of each chromosome. Faint, but nonetheless characteristic bands were present in the short arms of all seven chromosomes but in the long arms of only two of them (4A and 7A). Characteristic telomeric C-bands were present in the short arms of two of the seven chromosomes (1A and 5A). These characteristic banding patterns allowed for the identification of each of the seven chromosomes in the genome.

Gill (1987) established the C-banding patterns of the chromosomes of the A genome in *T. aestivum*. A comparison of the C-banding patterns of these chromosomes with those of *T. monococcum* reveals that the SAT chromosomes 1A and 5A have banding patterns that are different from the other chromosomes in the genome. Moreover, the comparison reveals similarities in banding patterns of chromosomes 2A, 3A and 7A in the cultivated polyploid wheats with their homologues in *T. monococcum*. The identity of one of the two remaining chromosomes in the *T. monococcum* genome was established as 4A by analyzing the C-banding patterns of a 29-chromosome segregant in *T. turgidum* which possessed 13 pairs of *T. turgidum* chromosomes, a 4A chromosome from this species, and a pair of 4A chromosomes from *T. monococcum* var. *boeoticum* (Figs. 12 and 13). The other chromosome was almost identical in relative length and arm ratio to chromosome 6A of cultivated polyploid wheats but differed from the latter with respect to its C-banding pattern. Consequently, a designation of the chromosomes of *T. monococcum* that is equivalent to the established nomenclature


a
2A
3A
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2A
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3A
2A
5A

Fig. 10. C-banded metaphase chromosomes of *T. monococcum*: (a) diploid line 89 ($2n = 2x = 14$), (b) autotetraploid of line 89 ($2n = 4x = 28$). (Magnification : 1300 X)

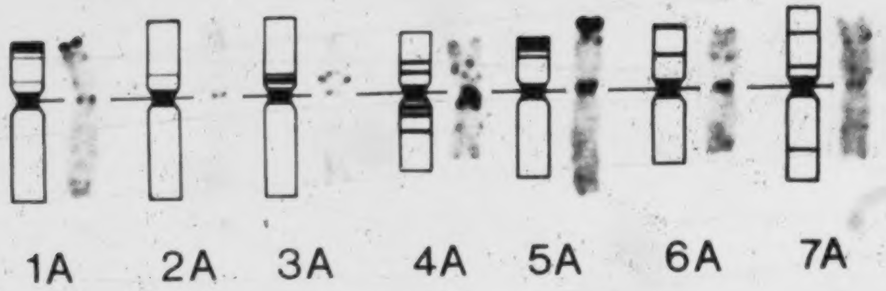

1A
2A
3A
4A
5A
6A
7A

Fig. 11. C-banded karyotype of *T. monococcum* line 89 (chromosome measurements are based on 15 complete C-banded metaphase spreads). (Magnification : 2000 X)

for their homologues in the polyploid wheats, seems feasible. The *T. monococcum* chromosomes are therefore designated accordingly.

ii) Specific

C-banding patterns of chromosomes carrying NORs

SAT CHROMOSOMES 1A and 5A: Analysis of nucleolar activity (Table 6) and *in situ* hybridization with a rDNA probe (Figs. 8 and 9) revealed that only two chromosomes in the genome were satellited and possessed NORs in the terminal segments of their short arms.

These two chromosomes possessed telomeric C-bands and were easily distinguishable from the other chromosomes. In all the metaphase cells analyzed, both of these chromosomes and their homologues lacked C-bands in their long arms whereas one of them possessed large telomeric C-bands in the short arm and the other had smaller C-bands in the corresponding region. In the majority of the metaphase cells that were studied, the chromosome with the large C-bands (with an arm ratio of 1.6) was slightly shorter than the other chromosome pair (with an arm ratio of 1.8). In addition, both of these chromosomes showed faint C-bands near the telomeric bands, with the band in one being larger than that in the other. Furthermore, in one of these chromosomes a very faint C-band was observed in the proximal region of the short arm.

In the cultivated polyploid wheats, chromosome 1A may or may not show a small C-band in the terminal region of the short arm. Chromosome 5A always shows a large C-band in the terminal segment of the short arm (Gill 1987). In addition, chromosome 1A of these wheats usually has two small bands in the long arm, one adjacent to the centromere and the other in the telomeric region. The long arm of chromosome 5A also possesses two small bands, one adjacent to the centromere and another in the distal half. On the basis of the differences in the C-banding patterns of the short arms of chromosome 1A and 5A of *T. aestivum*, it seems justifiable to designate the chromosomes with telomeric bands in *T. monococcum* accordingly: the one with the large terminal C-band as chromosome 5A and the other with the smaller telomeric C-band as chromosome 1A.

Different accesions of *T. monococcum* showed polymorphic variation in the size of the telomeric C-bands in the 1A pair of chromosomes (see Fig. 14). Similar variation might also exist in other accessions of *T. monococcum* for chromosome 5A. This variation is probably caused by corresponding differences in the number of copies of rRNA genes in these chromosome regions. The designations of SAT chromosomes as 1A and 5A therefore cannot be viewed as unequivocal until confirmed by further biochemical and molecular analyses.

C-banding patterns of chromosomes without NORs

CHROMOSOME 2A (arm ratio 1.3): One of the chromosomes in the genome revealed a very small faint band near the centromere in its short arm (Figs. 10 and 11). This feature enabled this chromosome to be distinguished from the others in the complement. On the basis of the similarity to chromosome 2A of cultivated polyploid wheats (Gill 1987) in length, arm ratio, and some C-bands, this chromosome in *T. monococcum* is designated as 2A. The characteristic C-band which is usually present in the proximal region of the long arm of chromosome 2A in polyploid wheats, is absent from its homologue in *T. monococcum*, and the small faint C-band in the short arm of this chromosome in *T. monococcum* is absent from its homologue in the polyploid wheats.

CHROMOSOME 3A (arm ratio 1.4): A prominent C-band located near the centromere and in the short arm could be seen in one of the chromosomes in the genome (Figs. 10 and 11). In good spreads the band was often observed to be composed of two small bands, the larger of which was located proximally. This feature allowed this chromosome to be distinguished from others in the complement. This chromosome is almost identical in morphology and C-banding pattern to chromosome 3A of polyploid wheats. In view of this identity it was therefore designated 3A.

CHROMOSOME 7A (arm ratio 1.2): One of the chromosomes in the genome showed a faint proximal C-band and a distal C-band in the short arm as well as a faint distal C-band in its long arm, allowing it to be discerned from other chromosomes in the complement (Figs. 10 and 11). The size, arm ratio, and some C-bands of this chromosome were similar to that of chromosome 7A of cultivated wheats. Therefore, it was designated it 7A. However, chromosome 7A of cultivated wheats and that of *T. monococcum* are different with respect to some C-bands. Chromosome 7A of cultivated polyploid wheats has faint, distally located C-bands and large telomeric C-bands in one or both arms. These telomeric bands were not observed in chromosome 7A of *T. monococcum*. The basis for the absence of these large telomeric bands in this chromosome of *T. monococcum* will be discussed in an ensuing section.

The C-banding patterns of the two remaining chromosomes (4A and 6A) of *T. monococcum* genome were not entirely equivalent to those of any of the chromosomes in the A genome of the polyploid wheats (Figs. 10 and 11). Therefore, they could not be designated as homologues of the chromosomes in the A genome of polyploid wheats.

The two undesignated chromosomes and their identification

One of these chromosomes was submetacentric (arm ratio 1.2) and showed two faint C-bands in the proximal half of the short arm and C-bands in the long arm that were close to each other and proximal to the centromere. These latter bands often fused to form one large C-band. The middle of the long arm also possessed a faint C-band. These features clearly distinguished this chromosome from others in the *T. monococcum* genome. The gross morphology and C-banding patterns of this chromosome are different from those of all the chromosomes in the A genome of polyploid wheats thus precluding its designation as being homologous with any of the chromosomes in the A genome. The necessity to establish the true identity of this chromosome will be discussed in the ensuing section.

The other chromosome was slightly submetacentric (arm ratio 1.1). It showed a faint C-band in the middle of the short arm and a faint C-band at the telomere of this arm. However, this telomeric band was much smaller than the telomeric C-bands observed in the short arms of the chromosomes designated as 1A and 5A. These features distinguished this chromosome from others in the genome. Although the size and arm ratio of this chromosome were similar to that of chromosome 6A of cultivated polyploid wheats, their C-banding patterns were different. Chromosome 6A of the polyploid wheats lacks the C-band in the middle of the short arm but shows up to four C-bands in the long arm. These bands were not observed in the long arm of this chromosome of *T. monococcum*. These C-banding differences precluded the designation of this chromosome as being homologous to 6A of the polyploid wheats. The basis for the identification one of these undesignated chromosomes is described in the ensuing section.

Identification of one of the undesignated chromosomes of *T. monococcum* as 4A

The results obtained by Dvořák (1983), Chen and Gill (1983), Rayburn and Gill (1985), and Naranjo *et al.* (1988), in polyploid wheats showed that the chromosomes that were initially designated as 4A and 4B should instead be assigned to the B and A genomes, respectively. This reallocation was approved at Workshop I of the Seventh International Wheat Genetics Symposium held in

Cambridge, England (Morris and Miller 1988). Chromosome 4A of the cultivated polyploid wheats, possesses multiple structural rearrangements (Chapman *et al.* 1976; Dvořák 1983; Naranjo *et al.* 1988). In addition to a differential staining of the centromeric region, it shows a large, distally located C-band in the long arm. Furthermore, this arm is marked by a distinct band near the centromere and two smaller C-bands. One of these bands is close to, and on the non-telomeric side, of the distally located large C-band. The other is in the telomeric region of this arm. The true identity of this undesignated chromosome in *T. monococcum* was determined by using a 29-chromosome segregant in *T. turgidum* with one 4A chromosome from *T. turgidum* and a pair of 4A chromosomes from *T. monococcum* var. *boeoticum*. This 29-chromosome segregant was an advanced generation plant derived from the cross *T. turgidum* var. *durum*♀ x *T. monococcum* var. *boeoticum*♂ (For detailed information on its mode of origin, see Joppa and Maan 1982). The C-banding patterns of the chromosomes in this segregant were compared with C-banding patterns of the chromosomes of the A and B genomes of the cultivated polyploid wheats.

The C-banding patterns of the chromosomes from a metaphase spread of this 29-chromosome segregant and the resultant composite karyotype are shown in Fig. 12 and 13, respectively. With the exception of one chromosome pair (4A) and one homologue of another pair (7A), all the other chromosomes showed C-banding patterns that were similar to those of their homologues in cultivars of *T. turgidum* and *T. aestivum*. The chromosome pair marked with a triangle in Fig. 12 and the chromosome similarly identified in Fig. 13 revealed a C-banding pattern that was different from that of the chromosome on its immediate left which is chromosome 4A of *T. turgidum*. Instead, this chromosome was identical in morphology and C-banding pattern to one of the two chromosomes that could not be identified in the genome of *T. monococcum*. The C-banding pattern of this undesignated

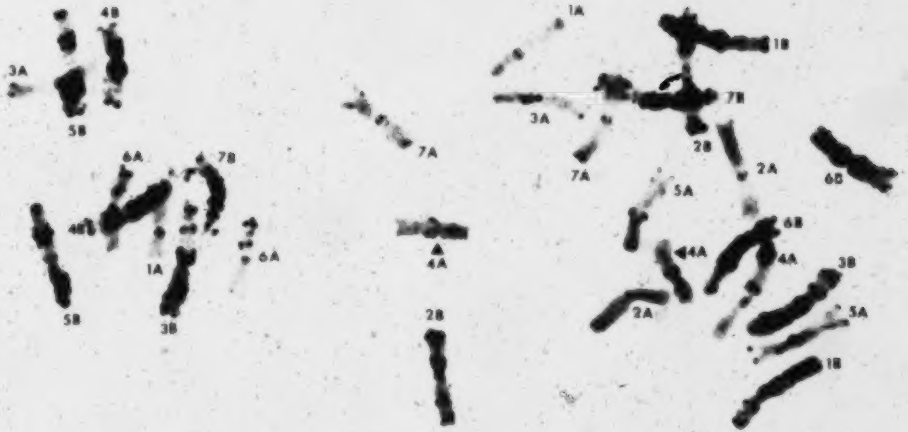

4B
3A
5B
6A
7B
4B
1A
6A
5B
3B
7A
4A
2B
1A
1B
3A
7B
7A
2B
2A
6B
5A
6B
4A
4A
3B
2A
5A
1B

Fig. 12. C-banded mitotic metaphase of a 29-chromosome segregant in *T. turgidum*. It has three 4A chromosomes, one from *T. turgidum* and two from *T. monococcum* var. *boeoticum*. Chromosomes from *T. monococcum* var. *boeoticum* are marked with triangles. (Magnification : 1300 X)

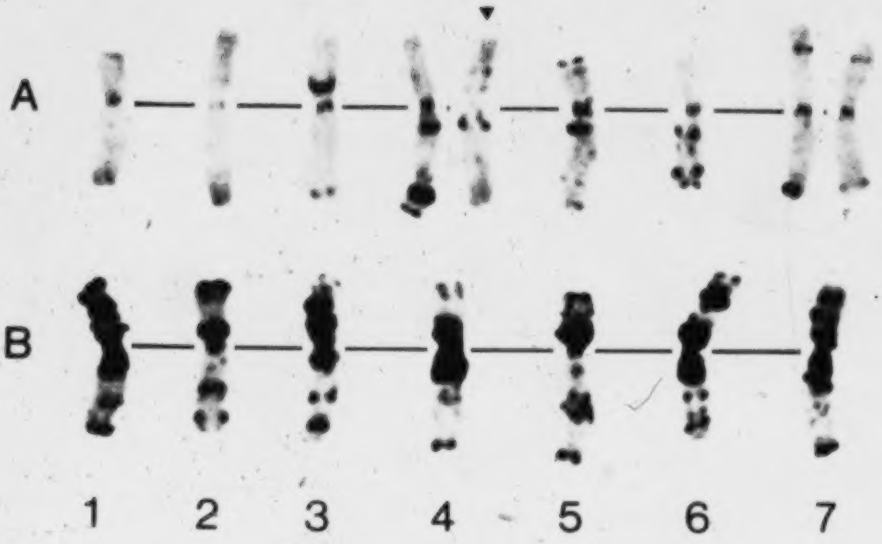


Fig. 13. C-banded karyotype of the 29-chromosome segregant in *T. turgidum*. It has three 4A chromosomes, one from *T. turgidum* and two from *T. monococcum* var. *boeoticum* (4A from *T. turgidum* on the left; 4A from *T. monococcum* var. *boeoticum* on the right). The karyotype also includes two 7A chromosomes, one from *T. turgidum* and the opther from *T. monococcum* var. *boeoticum*. (See following page for discussion of the differences between them).
(Magnification : 2000 X)

chromosome in *T. monococcum* appeared to partially resemble the chromosome 4A of polyploid wheats. Joppa and Maan (1982) in their phenotypic comparisons of the *T. turgidum* substitution line with this pair of *T. monococcum* chromosomes, and *T. turgidum* showed that, except for the GA_3 requirement for seed germination, a specific chromosome of *T. monococcum* var. *boeoticum* compensated well for the loss of chromosome 4A of *T. turgidum*. Compensation is known to occur only among chromosomes belonging to the same homoeologous group (Sears 1969). This implies that the *T. monococcum* var. *boeoticum* chromosome is homoeologous to chromosomes of group four of the *Triticinae*. Thus, one of the two chromosomes in *T. monococcum* that could not initially be identified was designated as 4A. The difference in C-banding patterns between this chromosome of *T. monococcum* and its partial homologue 4A of the polyploid wheats may be the result of structural rearrangements.

The process of elimination therefore permits the other chromosome in *T. monococcum* that could not be identified initially to be designated 6A. The difference in the C-banding patterns of this chromosome in *T. monococcum* and its putative homologue 6A in the polyploid wheats suggests that structural rearrangements may also have been involved in the formation of the latter.

Differences between chromosomes 7A of *T. monococcum* and *T. turgidum*

The karyotype in Fig. 13 shows a pair of 7A chromosomes with different C-banding patterns. The chromosome on the left is from *T. turgidum*. It shows a large terminal C-band in the long arm and a fainter, distally located band in the short arm. This pattern is similar to that of chromosome 7A from other cultivars of polyploid wheats (Gill 1987). However in the homologue on the right, the distal region of the long arm of this chromosome possesses a faint, subterminal band and not a large terminal one. This chromosome resembles 7A of *T. monococcum*. The difference in C-banding patterns in the long arms of these two chromosomes indicates that at least the distal part of the long arm of the chromosome from *T. turgidum* may not be completely homologous with chromosome 7A from *T. monococcum*. The meiotic pairing data of Joppa and Maan (1982) implied that not only chromosome 4A of *T. turgidum*, but additional genetic material from *T. monococcum* var. *boeoticum* is present in the chromosome substitution line. The C-banding pattern of chromosome 7A in *T. turgidum* indicates that the additional genetic material is present in the long arm of this chromosome.

General comments on C-banding in T. monococcum

The C-banding patterns of the chromosomes of the autotetraploid were identical to those described for the diploid, thereby confirming the identification of all chromosomes (Fig. 10).

Although the overall C-banding patterns and morphologies of the chromosomes of *T. monococcum* were similar to those of the chromosomes in the A genome of cultivated polyploid wheats, differences were observed in the locations and intensities of some of the C-bands in all the chromosomes except 3A. Structural rearrangements indicated by differences in chromosome morphology and C-banding patterns, may be present in chromosomes 1A, 5A, and 6A. The differences in chromosomes 2A and 7A of *T. monococcum* and cultivated polyploid wheats were primarily in band size.

Structural rearrangements may not necessarily account for the observed differences. They could be due to one or more mechanisms of amplification. On the basis of C-banding patterns, five of the seven chromosomes of *T. monococcum* were unequivocally designated as being homologous with specific chromosomes in the A genome of polyploid wheats. One chromosome in the diploid species, almost completely different in its banding pattern from any of the chromosomes of the A genome in the cultivated polyploid wheats, was designated as 4A. The process of elimination indicated that the other undesignated chromosome in the genome of *T. monococcum*, with a different C-banding pattern, may be 6A.

2. Identification of trisomics

Ag-NOR staining, *in situ* hybridization using an rDNA specific probe, and C-banding were used to identify the chromosome present in triplicate in each of the 18 primary trisomic lines as well as a primary trisomic plant in line 42, which may have been produced by trivalent shift (any mitotic and meiotic mechanism that gives rise to a trisomic for a particular chromosome in triplicate in a individual trisomic for a different (non-homologous) chromosome in the genome). The results obtained are summerized in Table 7. The C-banded mitotic metaphases of some of the different trisomics are shown in Fig. 14.

a. Identification of trisomics for chromosomes carrying NORs

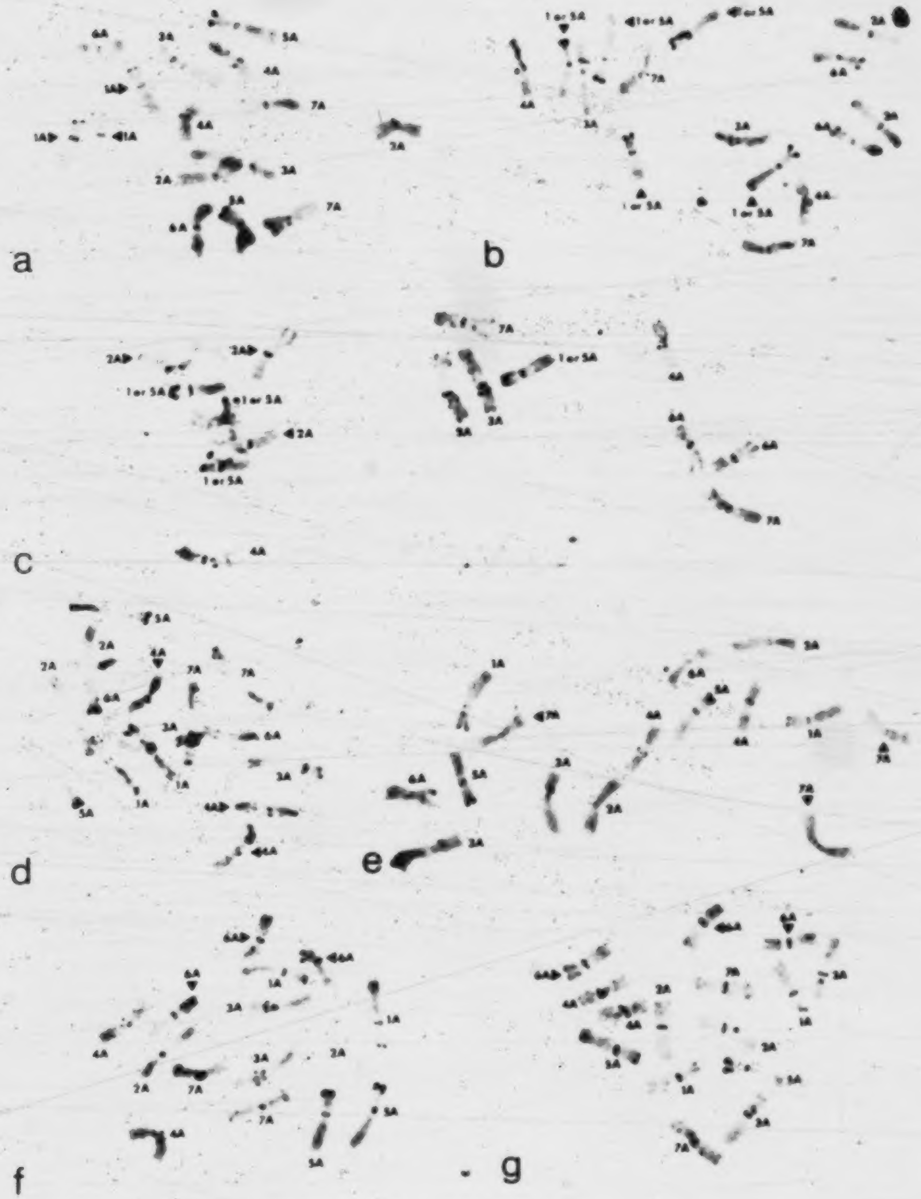
Line 89 of *T. monococcum* possessed four nucleoli at interphase. The rDNA probe hybridized to the terminal regions of the short arms of a maximum of four chromosomes in this diploid line (Table 6). Trisomics from six of the trisomic lines analyzed (7, 20, 28, 38, 50, 51), as well as an exceptional trisomic plant 41 from line 42, revealed as many as five interphase nucleoli (Table 6). Moreover, in each of these lines, the 26S rDNA probe hybridized to the terminal regions of the short arms of five chromosomes (Table 6; Figs. 8 and 9), indicating the presence of NOR loci on these chromosomes. These results indicated that each of the 6 lines, as well as plant 41, was trisomic for either of the two SAT chromosomes 1A or 5A. Furthermore, it appeared that the extent of hybridization of the rDNA to the NORs was heavier on chromosome 5A than on chromosome 1A (Figs. 8, 9 and 15). Similar observations have also been made by Gerlach *et al.* (1980) and Miller *et al.* (1983). The SAT chromosome present in triplicate in trisomics of line 50 showed

Table 7. Identity of the chromosomes present in triplicate in each of the *T. monococcum* trisomic lines.

Trisomic lines	Trisomic for chromosome
41, 50*	1A
3	2A
26,40, 52, 58	3A?
4, 6, 47	4A
28*, 51*	5A
8, 16, 29, 33, 36, 42, 57	6A
10, 22, 34, 39,	7A
7, 20, 38	1A or 5A

* Identified using an rDNA probe and *in situ* hybridization.

Fig. 14. C-banded metaphases of *T. monococcum* trisomics. (a) Trisomic for chromosome 1A from line 41. (b) Trisomic for chromosome 1A or 5A from line 7. (c) Trisomic for chromosome 2A from line 3. (d) Trisomic for chromosome 4A from line 6. (e) Trisomic for chromosome 7A from line 22. (f) and (g) Trisomics for chromosome 6A from line 16 and 36, respectively (critical chromosomes are marked with triangles). (Magnification : 1100 X)


a
b
c
d
e
f
g

light hybridization, strongly suggesting that they were trisomic for chromosome 1A. Since the SAT chromosome present in triplicate in trisomics of lines 28 and 51 showed heavy hybridization, they were deduced to be trisomic for 5A. The degree of hybridization in trisomics of lines 7, 20 and 38, with either of SAT chromosomes in triplicate, was variable and therefore precluded the unequivocal identity of the extra chromosome in these lines. Should the difference in labelling intensity for chromosomes 1A and 5A prove to be true for all accessions, it may be used as a criterion for distinguishing between trisomics for these two chromosomes. In all the trisomics analyzed from each of the six lines and trisomic plant 41, five chromosomes showed telomeric C-bands in their short arms (Fig. 14 a and b). This substantiates the contention that either chromosome 1A or 5A is present in triplicate in the trisomics from these six lines and the exceptional trisomic plant.

All the cells from a given plant showed a specific number of large telomeric C-bands in the short arms of chromosomes 1A and 5A. However, this number was found to vary among different plants within these trisomic lines. For a given plant trisomic for one of the SAT chromosomes, the number ranged from two to five. Fig. 14a shows a metaphase spread from the exceptional trisomic plant 41 with five SAT chromosomes. Two of these chromosomes reveal large telomeric C-bands and three possess telomeric C-bands that are small. This C-band constitution leads to the unequivocal conclusion that this plant is trisomic for chromosome 1A. Fig. 14b shows a metaphase chromosome complement with five large telomeric C-bands from a plant in line 7 which has to be trisomic for 1A or 5A. Fig. 14c shows a mitotic metaphase spread from line 3, trisomic for chromosome 2A, with four SAT chromosomes possessing large telomeric C-bands. This is in contrast to diploid line 89 in which only two of the four SAT chromosomes possess large telomeric C-bands (Fig. 10). This variation in the number of large telomeric C-bands can be attributed to at least one of the diploid parents of the trisomics possessing similar large-sized telomeric C-bands in the short arms of both chromosomes 1A and 5A. The polymorphic variation in the telomeric C-bands in the short arms of chromosomes 1A and 5A made it difficult to distinguish between trisomics for chromosomes 1A and 5A. This polymorphic variation in C-band size in chromosomes 1A and 5A might reflect a similar polymorphism for the copy number of rDNA genes in these chromosome regions. Although the results indicated that each of the six lines was trisomic for either of the two SAT chromosomes, additional genetic, cytogenetic, and biochemical analyses including the use of RFLP markers are necessary to verify the identification of the chromosome in triplicate in these lines.

b. Identification of trisomics for chromosomes not carrying NORs

In each of the remaining trisomic lines the observed number of interphase nucleoli per cell did not exceed four (Table 4). This indicated that the chromosome present in triple dose in the trisomics of these lines was neither 1A nor 5A. This was confirmed by the observation that in complete metaphase complements from trisomics of these lines, only four chromosomes showed *in situ* hybridization with an rDNA probe (Table 5; Figs. 8, 9, 15). This was further substantiated by the C-banding patterns of these chromosomes present in triplicate in the trisomics (Fig. 14). On the basis of a comparison of the C-banding patterns of the chromosomes in these trisomics (Fig. 14) with those of chromosomes 2A, 3A, 4A, 6A, and 7A in line 89 (Figs. 10 and 11) it was concluded that:

(i) Line 3 was trisomic for chromosome 2A.

(ii) Lines 4 and 6 were both trisomic for chromosome 4A.

(iii) Lines 8, 16, 29, 33, 36, 42, and 57 were trisomic for chromosome 6A. Two different C-banding patterns were observed for chromosome 6A in these lines. The C-banding pattern in one was identical to chromosome 6A of the diploid line 89; whereas the other differed by showing an additional prominent C-band in the short arm (Fig. 14. f and g). This polymorphism did not preclude identification of this chromosome in triplicate in these lines.

(iv) Lines 10 and 22 were trisomic for chromosome 7A. These trisomics could also be identified on the basis of their distinct phenotype viz. the small narrow heads (Fig. 19) and small narrow leaves (Fig. 20).

Although the chromosome present in triplicate in trisomics in lines 26, 40, 52 and 58 could not be identified cytologically, it is highly likely that they carry chromosome 3A in triple dose. This conclusion was based on the observation that these four lines each possessed a similar phenotype, distinct from that of all the other cytologically identified trisomic lines.

A new set of primary trisomics produced in the true-breeding diploid *T. monococcum* line 89 should avoid difficulties of chromosome identification because of polymorphism at some of the C-banding regions.

C. Concluding comments on C-banding

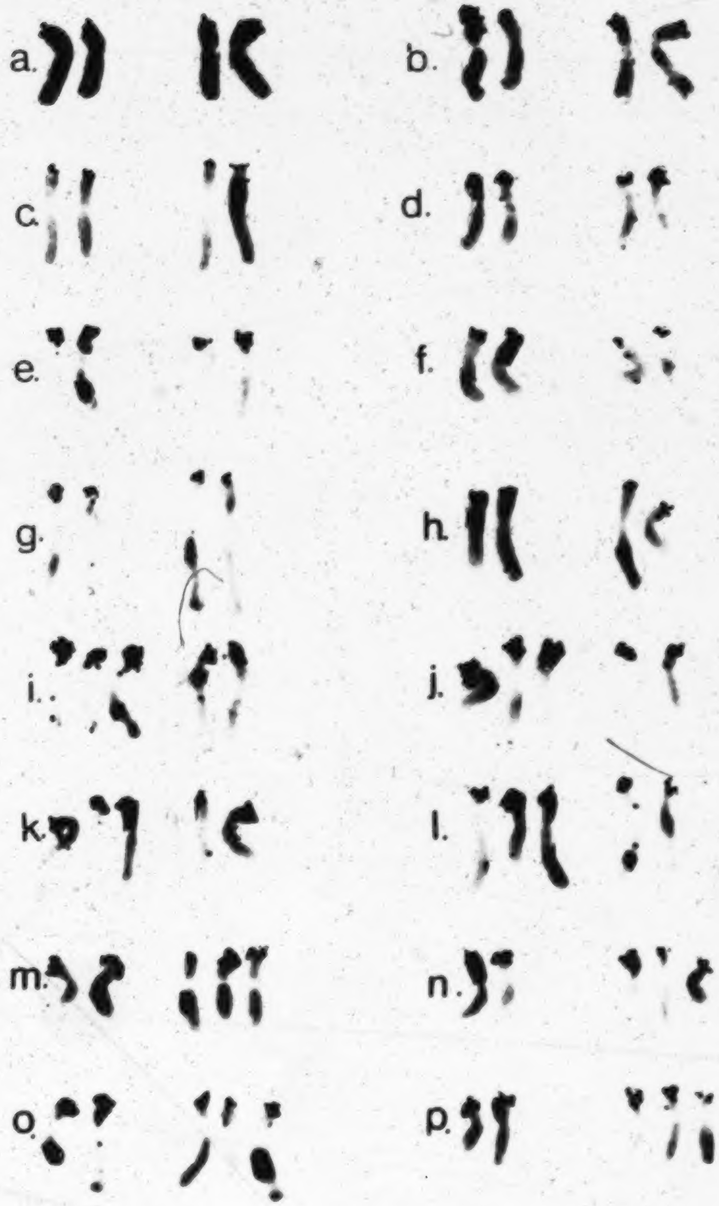
The C-banding technique identifies regions of constitutive heterochromatin in chromosomes (Pardue and Gall 1970; Jones 1970). In *T. monococcum*, as in most plant species (Linde-Laursen 1975; Gerlach and Peacock 1980; Endo 1986; Morris and Gill 1987), constitutive heterochromatin occurs at various sites throughout each chromosome, including centromeric and telomeric regions. With few exceptions, each chromosome possesses a unique C-banding pattern that permits its identification within a complement (Loidl 1983; Linde-Laursen 1986; Endo 1986; Gill 1987).

A comparison of the C-banding patterns of chromosomes in *T. aestivum* (Endo 1986; Fig. 3) and *T. monococcum* reveals that the C-bands at many sites in the A genome chromosomes in the bread wheat are larger than their corresponding homologous counterparts. This implies that the larger C-bands in *T. aestivum* have probably evolved by unequal crossing over since the origin of the polyploid wheats. Many of the constitutive heterochromatin regions in *T. monococcum* chromosomes are small. Therefore the C-banding technique required modification to permit the identification of chromosomes.

III. Location of 5S rRNA genes in *T. monococcum*

A 5S DNA probe was found to hybridize to the terminal regions of the short arms of four chromosomes in the diploid chromosome complement of root-tip cells of this species (Table 6; Fig. 8). This probe also hybridized to the short arms of the homologues of two chromosome pairs in meiocytes of diploids (Fig. 9). Therefore, two chromosome pairs in *T. monococcum* carry 5S rRNA genes. Moreover, a mixture of 26S rDNA and 5S rDNA probes also hybridized to the same regions of these four chromosomes (Table 6; Figs. 8 and 9). It was concluded earlier that the 18S and 26S rRNA genes were in the NOR regions of the two pairs of SAT chromosomes (1A and 5A) in *T. monococcum* (Gerlach *et al.* 1980, Miller *et al.* 1983). On the basis of these observations it was concluded that the 5S rRNA genes are located on the SAT chromosomes and are in juxtaposition to the NOR regions. *In situ* hybridization studies with trisomics using 5S rDNA and a mixture of 26S rDNA probes confirmed the above observations and conclusions (Table 6; Figs. 8 and 9). Since recombination studies were not carried out, it was impossible to determine the degree of linkage between the 5S rRNA and NOR genes. This study was the first in *T. monococcum* to localize 5S rRNA genes and confirm the findings of Dvořák *et al.* (1989) that these genes are on chromosomes 1A and 5A.

Fig. 15. Extent of *in situ* hybridization with 5S rDNA, 26S rDNA and a mixture of the two probes in SAT chromosome pairs 1A and 5A in diploids (a, b, c, d), trisomics whose chromosomes in triplicate do not carry NORs (e, f, g, h) and trisomics whose chromosomes in triplicate carry NORs (i, j, k, l, m, n, o, p). In each set of SAT chromosome pairs, the pair on the left is heavily labelled and the one on the right is lightly labelled. Hybridization in each of the 16 sets involved the following probes; (a) 26S rDNA, (b) mixture of 5S rDNA and 26S rDNA, (c) 26S rDNA, (d) mixture of the two probes, (e, from TT3) 5S rDNA, (f, from TT8) 26S rDNA, (g, from TT16) 26S rDNA, (h, from TT16) 26S rDNA, (i, from TT28) mixture of the two probes, (j, from TT51) mixture of the two peobes, (k, from TT28) mixture of the two probes, (l, from TT28) mixture of the two probes, (m, from TT50) 5S rDNA, (n, from TT50) 5S rDNA, (o, from TT50) 26S rDNA, and (p, from TT50) 26S rDNA. (Magnification : 1200 X)


a.
b.
c.
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i.
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m.
n.
o.
p.

Other studies on the location of 5S rRNA genes in wheat have been carried out in *T. aestivum*. Appels *et al.* (1980) localized the major 5S rRNA genes to chromosome 1B, at a site distinct from the NOR region, but close and distal to it. Kota and Dvořák (1986) mapped the 5S rRNA genes on chromosome 5B to a single site on the *p* arm. Lassner and Dvořák (1985) suggested that chromosomes 5A and 5D may also carry 5S rRNA genes. Scoles *et al.* (1987) obtained unequivocal evidence for the location of 5S rRNA genes on chromosomes 1B, 1D, and 5B. They confirmed the finding of Appels *et al.* (1980) that the 5S rRNA genes on chromosome 1B are at a single site closely linked to the Nor-B1 locus.

In most plant (Wimber *et al.* 1974; Appels *et al.* 1982; Doyle 1988) and animal species (Aloni *et al.* 1971; Pardue *et al.* 1973; Henderson *et al.* 1976; Leon and Kezer 1978) 5S rRNA genes are separated from the NOR genes, and are usually on different chromosomes. For example, in *Xenopus laevis* (*2n=36*) they are located near the telomeres of 26 chromosomes, wheareas only one chromosome pair possesses NORs (Pardue *et al.* 1973). Aside from wheat, only in rye (Appels *et al.* 1980), yeast (Bell *et al.* 1977), and *Dictyostelium* spp. (Maizels 1976) are the 5S rRNA and NOR genes closely linked.

The significance, if any, of the complete or close linkage vs. loose or non-linkage of the 5S rRNA and NOR genes is unknown. Since the RNAs specified by these genes are required in approximately the same amounts for ribosomal formation, one advantage of them being in juxtaposition may be that a common regulatory mechanism would ensure such equality.

IV. Cytological behaviour

A. Primary trisomics

Meiotic chromosome behaviour in the diploid progenitor of the aneuploids was observed to be completely normal (Fig. 6a). Chromosome behaviour in the different trisomic lines was studied at MI and AI in 100 microsporocytes each (Table 8; Fig. 16), which are described below.

1. Metaphase I

Observations of 490 bivalents in 70 meiocytes at MI in diploids showed that 15 of these associations were open with the remainder being closed and exhibiting terminalized chiasmata. Basically identical results were observed in the meiocytes of different trisomic lines. Of three hundred and sixty bivalents in 60 meiocytes at MI in trisomics, 12 were open bivalents and 348 closed. The open bivalents were attributed to asynapsis or desynapsis in one arm of the bivalent. Premature disjunction in an arm of a bivalent may also have caused it to be open. On the basis of these observations it was concluded that, on the average, there were two reciprocal exchanges and chiasmata per homologous chromosome pair, with one exchange and chiasma occurring in each arm of each bivalent. Observations of bivalents at D support this conclusion as do the studies of Kuspira *et al.* (1986b) on autotriploids in *T. monococcum*. Moreover, on rare occasions in plants trisomic for chromosome 7A, one of the three longest chromosomes in this species, a bivalent at D appeared to have three chiasmata.

The chromosome in triplicate in primary trisomics may exhibit one of three different associations at D-MI: 3Is, II+I, or III (Belling and Blakeslee 1924; Dawson 1962; Khush 1973). The frequencies of the three associations in a population of meiocytes depend on the size of the chromosome present in triplicate, location and incidence of occurrence of reciprocal exchanges and chiasmata, the extent of chiasmata terminalization and genetic factors (Einset 1943; Rick and Barton 1954; Dawson 1962; Vari and Bhowal 1986). Three univalents were not observed at D-MI in the meiocytes of any of the primary trisomics in *T. monococcum*. It should be noted that 3Is at D-MI have also not been observed in *L. esculentum* (Rick and Barton 1954) and *L. pedunculatus* (Chen and Grant

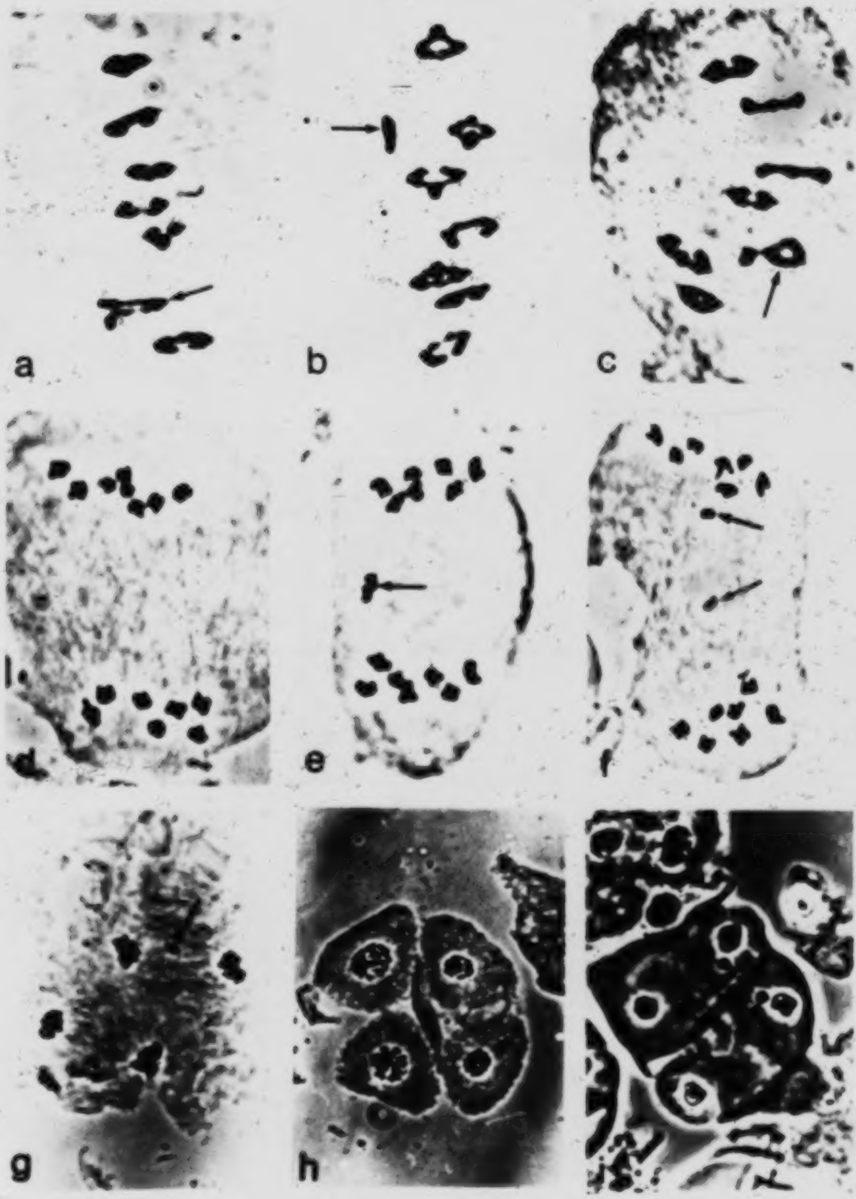
Table 8. Meiotic behaviour of chromosomes in 100 microsporocytes at metaphase I and at anaphase I in primary trisomics of *T. monococcum*.

Trisomic for chromosomes	Trisomic lines	Metaphase I associations			Anaphase I associations	
		7II+1I	6II+1III	Ave.% III	7 - 8	Others
1A	50	51	49	49	98	2(7+1C* - 7+1C)
2A	3	42	58	58	99	1(7 - 7, 1L**)
3A?	26	54	46	47.3	99	1(7 - 7, 1L)
	40	55	45		100	0
	52	52	48		100	0
	58	50	50		100	0
4A	4	51	49	49	100	0
	6	51	49		99	1(7+1C - 7+1C)
5A	28	48	52	51.5	99	1(7 - 7, 1L)
	51	49	51		99	1(7 - 7, 1L)
6A	8	48	52	55.6	100	0
	16	43	57		99	1(7+1C - 7+1C)
	29	48	57		100	0
	33	42	58		99	1(7 - 7, 1L)
	36	41	59		99	1(7 - 7, 1L)
	42	47	53		100	0
	57	48	52		100	0
7A	10	39	61	62.5	100	0
	22	32	68		100	0
	34	38	62		100	0
	39	41	59		100	0
1A or 5A	7	55	45	52.7	100	0
	20	42	58		99	1(7+1C - 7+1C)
	38	45	55		100	0

*C = chromatid of a univalent that divided equationally.

** L = lagging univalent with two chromatids.

Fig. 16. MI associations and AI distributions in primary trisomics of *T. monococcum*. (a) 6IIs + 1 V-shaped III (arrow). (b) 7IIs + 1I (arrow). (c) 6IIs + 1 frying-pan shaped III (arrow). (d) 7 - 8 distribution of chromosomes at AI. (e) 7 - 7 distribution of homologues plus a lagging I (arrow) at AI. (f) 7 - 7 distribution of homologues plus equational division of the I (arrows) at AI. (g) AII - telophase II with a lagging I in one of the secondary meiocytes. (h) Normal tetrad (no micronuceli). (i) Tetrad with micronuclei (arrows) in two of the four microspores.(Magnification : 700 X)


a
b
c
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f
g
h

1968a). They have seldom been observed in the meiocytes of other diploid plant species (Einset 1943; Myers 1944; Tsuchiya 1960; Manga 1976).

With the exception of the trisomics for chromosome 7A, the frequencies of cells with 7II + 1I and 6II + 1III were found to be present in approximately equal proportions (Table 8; Fig. 16) in other trisomics. This is not unexpected if, on the average, there were two reciprocal exchanges and chiasmata per bivalent, with one exchange and chiasma in each arm. Once two given homologues of a trivalent engage in a reciprocal exchange and chiasma formation in one arm, any two of the three homologues in the association may be involved in the second reciprocal exchange and chiasma formation in the other arm with equal frequency. If the same two chromosomes are involved in both exchanges, a II+I association will be formed. If one of the two homologues involved in the first reciprocal exchange, engages in the second exchange with the third chromosome in the association, a III will result. The proportions of II+Is and IIIs in the various *T. monococcum* trisomics were found to be different from those obtained in all other diploid plant species. For example, in *L. pedunculatus* (Chen and Grant 1968a) II+Is were found to be more common than IIIs, being present in 73.3 and 14.7% of the meiocytes, respectively. In all other species the reverse is true. For example, in *Z. mays* (Einset 1943) and *H. spontaneum* (Tsuchiya 1960), II+Is and IIIs were present with frequencies of 24.3 and 55%, 11.2 and 88.8%, respectively.

Trisomics for chromosome 7A in *T. monococcum* were observed to form II+Is and IIIs in an approximately 1:2 ratio (37.5 and 62.5% frequencies). This implied that reciprocal exchange and chiasma frequency was greater in the trivalents in these trisomics than in those trisomic for the other six chromosomes. Support for this conclusion was obtained from the observation of frying-pan trivalent configurations, which require more than two reciprocal exchanges. Chromosome 7A is one of the longest chromosomes in the genome (Table 5), and therefore, if there is a relationship between chromosome length and frequency of crossing over this result is not unexpected. However, such a relationship between II+Is and IIIs should also occur in trisomics for chromosomes 2A and 3A which are longer than chromosome 7A and are the two longest chromosomes in the genome (Table 5).

Of the four possible configurations (chain, frying-pan, Y-shaped and triple-arc) that trivalents can assume at D-MI (Fig. 2), in all the trisomics except those for chromosome 7A, only chain configurations were present in the form of a V, which always co-oriented convergently at MI (Fig. 16 a). These results were different from those obtained in other species. For example, in *D. stramonium* (Belling and Blakeslee 1924), *H. spontaneum* (Tsuchiya 1960) and *L. esculentum* (Khush 1973) all four configurations were observed with the chain and frying-pan types being much more common than the latter two (Table 1). In *P. typhoides* (Manga 1976) and *P. americanum* (Vari and Bhowal 1986) triple-arc configurations have not been observed nor reported, and, as in other diploid plant species, chain and frying-pan configurations are the ones most commonly observed (Table 1). In *T. monococcum*, trisomics for chromosome 7A formed V-shaped, frying-pan and Y-shaped trivalent configurations (Fig. 16) in 86%, 12%, and 2% of the meiocytes, respectively. These results are similar to those found in trisomics of the two *Pennisetum* species. In view of the fact that chromosome 7A is one of the longest chromosomes in the *T. monococcum* genome, the results that were obtained with lines trisomic for this chromosome are not unexpected. Since at least two reciprocal exchanges occur per bivalent and trivalent, and one in each arm, the rare Y-shaped trivalents in most, if not all cases, are probably derived from frying-pan types by precocious terminalization of the chiasma in the arm in which only two of the three homologues exchanged homologous segments.

2. Anaphase I

The segregation of chromosomes at AI is determined primarily by the orientation and co-orientation of their centromeres at MI. Although univalents can behave in a variety of different ways at AI (Sears 1952; Darlington 1957), in the vast majority of meiocytes of *T. monococcum* trisomics, they usually segregated to one or the other pole at AI (Table 8). All the meiocytes with 6II+1III at MI revealed a 7 - 8 distribution of chromosomes (Table 8; Fig. 16d). Most of the AI segregations in meiocytes with 7II+1I at MI were also 7 - 8. In approximately 1% of the meiocytes in which the chromosome in triplicate formed a II+I associations at MI, two other types of distributions were observed (Table 8, Fig. 16e and f). On rare occasions, the lagging univalent or its chromatids failed to segregate, thereby giving rise to micronuclei (Fig. 16i). None of the other patterns of behaviour of univalents, including misdivision to form isochromosomes and telocentrics were observed. Chromosomes from bivalent associations segregated randomly in a regular (1:1) manner. The homologues of V-shaped trivalents, which always co-oriented at MI, segregated randomly in a 2:1 fashion. Chromosomes from frying-pan and Y-shaped trivalents, which co-orient indifferently at MI, usually segregated 1:1 with the false univalent behaving like a lagging univalent or occasionally segregating randomly in a 2:1 manner. Occasionally, the univalent divided equationally and the chromatids segregated to opposite poles (Fig. 16f). Regardless of the mode of behaviour of univalents in primary trisomics of *T. monococcum*, *n* and *n+1* meiotic products were produced in approximately equal proportions. The paucity of trisomic progeny in reciprocal crosses between trisomics and diploids and in self-fertilizations of trisomics may be attributed to post meiotic-factors.

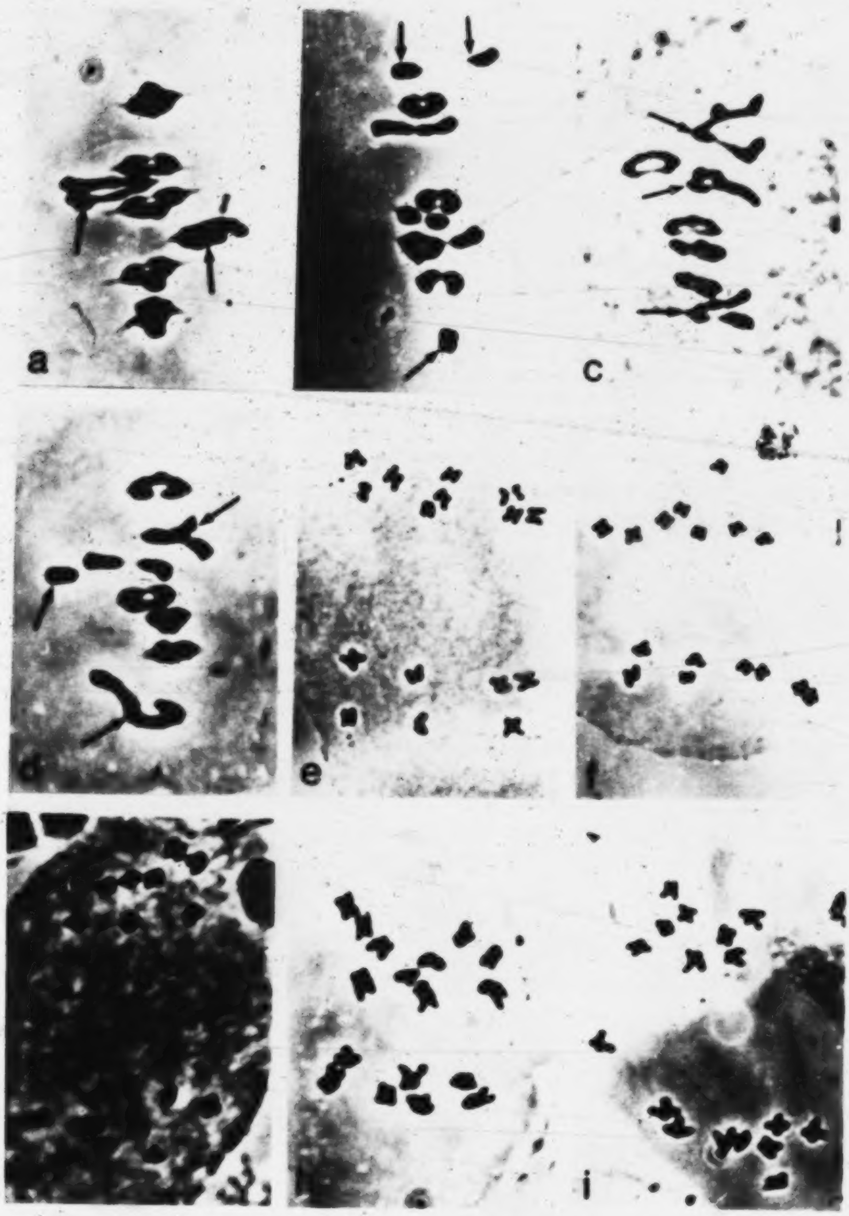
B. Double and triple trisomics

Table 9. Meiotic chromosome behaviour in double and triple trisomics of *T. monococcum*.

Aneuploids	Metaphase I				Anaphase I	
	No. of meiocytes observed*	Configurations			No. of meiocytes observed*	Distributions
		I	II	III		
Double trisomics	2	3	5	1 (V-shaped)	6	8 - 8
$2n=2x+1+1=16$	4	1	6	1 (V-shaped)	2	7 - 9
	6	0	5	2 (V-shaped)	1	7 - 7 (2L)
Triple trisomics	6	0	4	3 (V-shaped)	8	8 - 9
$2n=2x+1+1+1=17$	3	0	4	3 (2V + 1 frying pan)	6	7 - 10
	2	3	7	0	1	8 - 8 (1L)
	3	1	5	2 (V-shaped)		

* Of several 100 meiocytes examined at MI and AI, the numbers of discernible meiocytes are as indicated.

Fig. 17. MI associations and AI distributions in double and triple trisomics of *T. monococcum*. (a) MI in a double trisomic with 5IIs + 2IIIs (arrows). (b) MI in a double trisomic with 5IIs + 1III (arrow) + 3I (arrows). (c) MI in a triple trisomic with 4IIs + 3IIIs (arrows). (d) MI in a triple trisomic with 5IIs + 2IIIs (arrrows) + 1I (arrow). (e) AI in a double trisomic with a 7 - 9 chromosome distribution. (f) AI in a double trisomic with an 8 - 8 chromosome distribution. (g) AI in a double trisomic with a 7 - 8 chromosome distribution plus equational segregation of one univalent (arrow). (h) AI in a triple trisomic with a 7 - 10 chromosome distribution. (i) AI in a triple trisomic with an 8 - 8 chropmosome distribution plus a lagging I.(Magnification : 700 X)


a
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f
i

The results of studies of meiotic chromosome behaviour in these aneuploids are presented in Table 9 and Fig. 17. Aneuploids with 16 chromosomes were classified as double trisomics on the basis of the observation that some of their meiocytes formed five bivalents and two trivalents (Fig. 17a). Those with 17 chromosomes were determined to be triple trisomics on the strength of the observation that some of their microsporocytes formed four bivalents and three trivalents (Fig. 17c). A limited number of meiocytes were observed at AI (Fig. 17e, f, g, h, i), indicating that a majority of the secondary meiocytes and meiotic products possessed unbalanced chromosome numbers.

V. Phenotypes of diploids and trisomics of *T. monococcum*

A. Primary trisomics

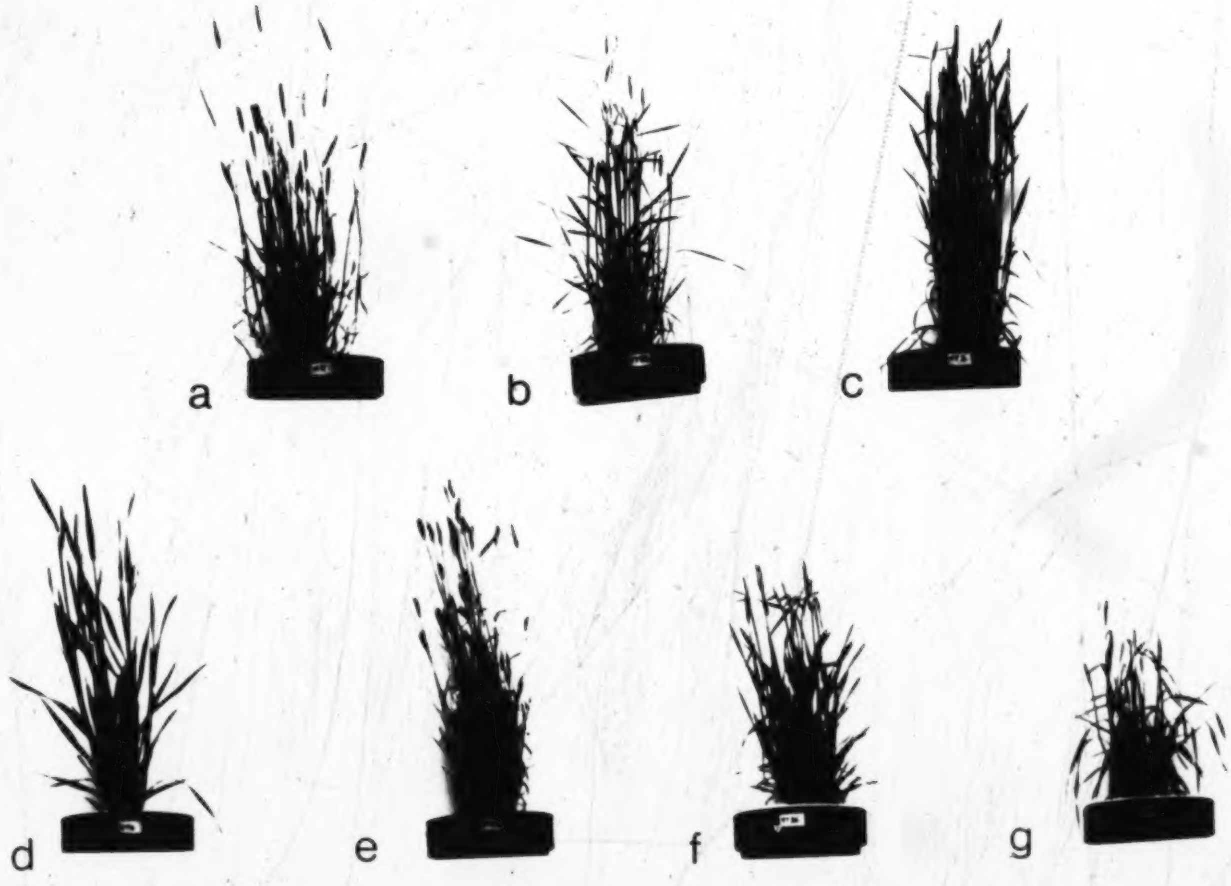
1. General

The phenotypes of the diploid progenitors and the various trisomics were determined for five quantifiable morphological characters and heading date over a four-year period beginning in 1986. The general plant, head, and leaf phenotypes are shown in Figs. 18, 19, and 20, respectively. The range and average phenotype for each character in trisomics in a given trisomic line were found to be very similar in the different years. The results were pooled and are presented in Table 10.

Consistent with the observations made in most diploid plant species, viz; in *Datura* (Blakeslee and Belling 1924), tomato (Lesley 1932; Rick *et al.* 1964), spinach (Janick *et al.* 1959), barley (Tsuchiya 1960, 1967), oats (Rajhathy 1975), rice (Khush *et al.*1984), pearl millet (Vari and Bhowal 1986), the *T. monococcum* trisomics were found to differ phenotypically from their diploid progenitors. Specifically, all of them were less vigorous, smaller (shorter), and slower growing. They produced fewer tillers and spikelets per head, had smaller leaves, and headed,

Fig. 18. General plant morphology of T. *monococcum* primary trisomics and their diploid progenitor. (a) Diploid. (b) Trisomic for chromosome 1A. (c) Trisomic for chromosome 2A. (d) Trisomic for chromosome 4A. (e) Trisomic for chromosome 5A. (f) Trisomic for chromosome 6A. (g) Trisomic for chromosome 7A.

* Trisomics for chromosome 3A were lost before they were photographed.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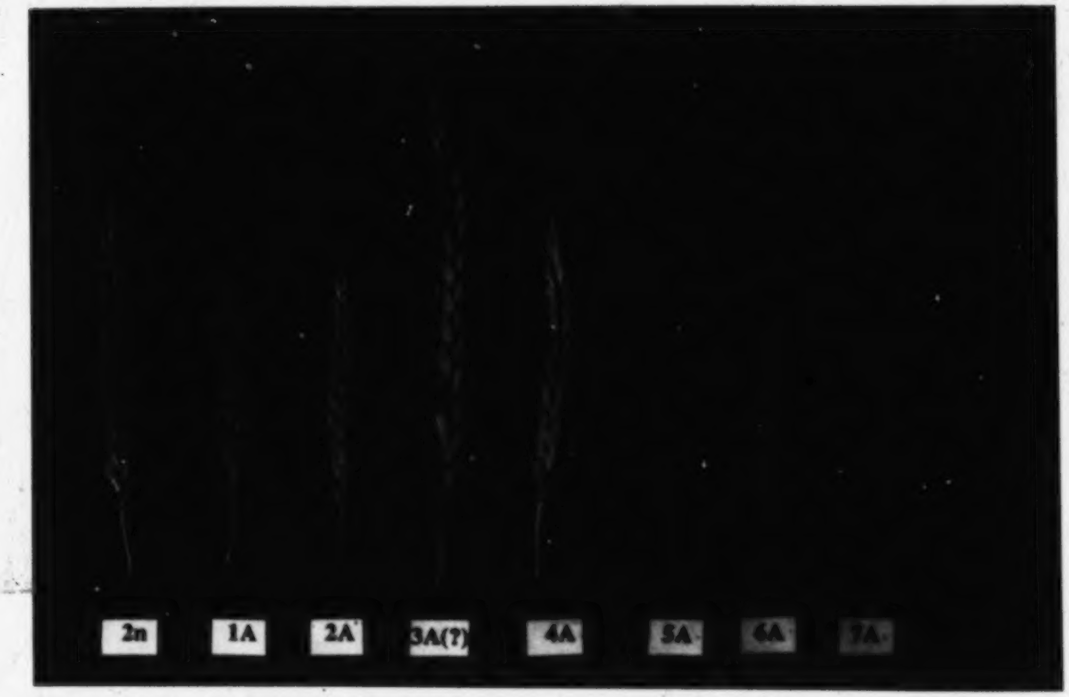
2n
1A
2A
3A(?)
4A
5A
6A
7A

Fig. 19. Heads of diploid line 89 and the seven primary trisomics of *T. monococcum*.

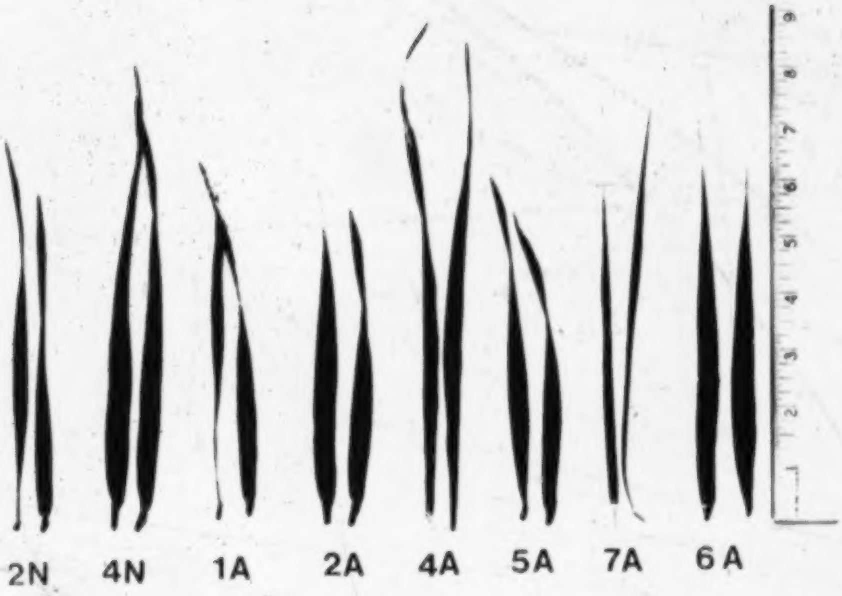
2N
4N
1A
2A
4A
5A
7A
6A
1
2
3
4
5
6
7
8
9

Fig. 20. Leaves of diploid line 89, autotetraploid line 89, and the six primary trisomics of *T. monococcum*. *Trisomics for chromosome 3A wre lost before their leaves were photographed.

Table 10. The range and average phenotypes for different morphological characters of the trisomics and diploid *T. monococcum*.

Chromosome in triplicate	Trisomic line*	Characters											
		Plant height(Cm)		Head length(Cm)		No. of spikelets per head		Flag leaf index (L/W) (Cm)		Heading date (days)		No. of tillers per plant	
		Range	Mean	Range	Mean	Range	Mean	Mean	Ratio(C/W)	Range	Mean	Range	Mean
1A	50	67.2 - 72.9	70.6	3.7 - 4.7	4.3	15 - 17	16.4	8.6/0.5	17.2	98.0 - 104.0	102.0	9 - 12	11.2
2A	3	78.1 - 85.0	83.7	5.0 - 5.6	5.3	19 - 21	19.6	11.2/0.9	12.4	10.1 - 107.0	104.8	10 - 13	12.4
3A?	26	72.2 - 78.3	75.1	11.2-12.4	11.7	27 - 29	28.0	12.8/0.8	16.1	96.0 - 99.0	97.4	9 - 13	12.8
	40	73.0 - 79.5	76.2	10.9-12.4	11.6	27 - 30	28.4	12.5/0.8	15.6	95.0 - 98.0	96.9	9 - 15	13.2
4A	4	68.0 - 75.6	72.4	7.1 - 7.7	7.4	25 - 27	25.6	16.0/0.9	17.7	94.0 - 99.0	95.3	7 - 11	9.6
	6	70.3 - 78.6	74.7	7.3 - 7.8	7.5	25 - 27	25.8	15.6/0.8	19.5	95.0 - 100.0	96.4	8 - 11	9.6
5A	28	68.9 - 74.0	71.9	4.2 - 5.0	4.7	19 - 21	20.2	9.7/0.7	13.6	97.0 - 102.0	100.1	8 - 10	9.3
	51	70.1 - 75.6	72.6	4.2 - 5.3	4.8	18 - 19	18.5	9.1/0.7	13.0	98.0 - 104.0	101.8	9 - 13	11.3
6	8	72.1 - 79.8	76.1	4.9 - 5.6	5.3	19 - 21	20.4	10.5/0.9	11.7	103.0 - 109.0	107.3	9 - 13	11.3
	16	70.1 - 75.6	72.6	5.3 - 6.1	5.7	19 - 20	19.6	11.3/0.9	12.4	101.0 - 107.0	105.3	10 - 13	11.5
	29	75.5 - 81.8	79.1	4.3 - 5.0	4.7	17 - 19	18.4	9.1/0.7	13.0	106.0 - 110.0	109.1	11 - 14	12.2
	33	78.1 - 85.3	82.1	4.5 - 5.2	4.8	17 - 19	18.6	10.1/0.9	11.2	104.0 - 109.0	106.4	8 - 12	10.1
	36	75.1 - 82.4	78.1	4.5 - 5.2	5.0	18 - 20	19.2	11.2/0.9	12.4	104.0 - 107.0	105.5	9 - 12	11.2
	42	75.1 - 83.6	78.7	4.9 - 5.8	5.3	21 - 22	21.1	12.0/1.0	12.0	105.0 - 109.0	107.8	8 - 11	9.7
	57	74.0 - 78.7	76.8	5.0 - 5.6	5.3	18 - 19	18.5	10.7/0.8	11.8	103.0 - 110.0	106.9	8 - 12	10.3
7A	10	61.9 - 68.2	64.1	4.9 - 5.2	5.2	14 - 15	14.8	10.7/0.4	26.8	109.0 - 115.0	113.6	8 - 11	9.6
	22	62.3 - 69.8	66.4	5.1 - 5.4	5.3	14 - 16	15.2	16.1/0.6	26.8	115.0 - 120.0	118.2	9 - 12	9.9
1A or 5A	7	76.6 - 81.9	79.2	5.4 - 6.2	5.8	16 - 18	17.4	12.3/0.7	17.6	99.0 - 106.0	104.0	8 - 12	10.2
	20	63.5 - 79.5	67.6	4.6 - 5.3	4.8	20 - 23	21.3	8.1/0.5	16.2	102.0 - 107.0	105.3	9 - 13	10.8
	38	62.3 - 69.8	66.4	4.5 - 4.8	4.8	18 - 20	19.4	8.5/0.6	14.2	103.0 - 107.0	105.8	11 - 15	13.1
Diploid		105.6-111.1	108.4	7.2 - 8.8	7.2	24 - 26	25.0	15.0/0.8	18.8	77.0 - 83.0	81.0	14 - 19	16

* No data on trisomics in lines 34, 39, 47, 52, and 58 which were lost early in the study.

flowered and matured later than the diploid lines. Some of them were highly sterile and others moderately so, unlike the diploids which were fully fertile (Table 12).

2. Specific

The trisomics in eight of the 25 lines obtained could be placed into two phenotypically distinct groups. Unlike the diploids and trisomics of all the other lines, trisomic from lines 10, 22, 34, and 39 had a distinct phenotype viz., small narrow heads (Fig. 19) and small narrow leaves (Table 10; Fig. 20). Cytological analysis revealed these lines to be trisomic for chromosome 7A. Trisomics from lines 26, 40, 52, and 58 produced larger heads than those of the diploids and of trisomics from all other lines (Table 10; Fig. 18). Moreover, their heads had a distinct non-*T. monococcum* phenotype. These were the four lines that were eventually lost because of very low fertility and failure to produce trisomics. None of the other trisomic lines possessed the phenotypic characteristics of the trisomics of these four lines. On the basis of their distinct phenotype together with the failure to cytologically identify a line trisomic for 3A, it was proposed that the trisomics in these four lines carried chromosome 3A in triple dose. Thus, of the series of seven primary trisomics in *T. monococcum*, only two produced distinct morphological features on the basis of which they can be distinguished. These results were inconsistent with those in *Datura* (Blakeslee 1922), spinach (Janick *et al.* 1959), snapdragons (Sampson *et al.* 1961), barley (Tsuchiya 1960), pearl millet (Manga 1976), rice (Khush *et al.* 1984), beets (Romagosa *et al.* 1986) and most diploid plant species, in which all or most of the different trisomics can be distinguished morphologically. The reasons why only two of the primary trisomics in *T. monococcum* produced distinct phenotypes are not known. The results observed in *T. monococcum* are similar to those in *Z. mays* in which only three of the ten trisomics can be identified morphologically from the other trisomics (Rhoades and McClintock 1935) and had extensive genetic duplication (Helentjaris *et al.* 1988;

Wendel *et al.* 1989). Therefore, the lack of morphological distinction among most of the *T. monococcum* trisomics may be attributed, at least in part, to duplication of genetic information.

The trisomics for chromosome 3A and 7A express distinct head and leaf head phenotypes, respectively. This implies that at least one gene for each of these characters resides on these chromosomes. The gene on chromosome 3A may be a hypomorph, whereas the genes on chromosome 7A may be suppressors of normal leaf and head development. Irrespective of the ability to identify trisomics morphologically, cytological analyses are still required for positive identification of all trisomics except those for trisomic 7A.

Comparisons with the diploid lines 68 and 89, which are phenotypically very similar for all of the characters studied, revealed the following main phenotypic features for each of the seven primary trisomics in *T. monococcum*;

TRISOMICS FOR CHROMOSOME 1A
-Normal growth habit, but ~ 35% shorter.
-Heads normal shape, but ~ 40% shorter.
-Number of spikelets per head was reduced by ~ 40%.
-Leaves were reduced in size by about 40%.
-Heading date ~ three weeks later.
-~ 35% fewer tillers.
-Highly sterile (96%).
-These features distinguish these trisomics from diploids but not from other trisomics.

TRISOMICS FOR CHROMOSOME 2A
-Normal growth habit with ~ 25% reduction in height.
-Heads possessed a *T. monococcum* phenotype, but were ~ 25% shorter.
-Number of spikelets per head was reduced by ~ 20%.
-Leaves were ~ 25% smaller.
-Heading date ~ three weeks later.

- Tillering was reduced by about 25%.
- Fertility was reduced by ~ 60%.
- These features permit easy distinction of these trisomics from diploids but not from other trisomics.

TRISOMICS FOR CHROMOSOME 3A?

- Normal growth habit with height reduced by ~ 35%.
- Heads were atypical, possessed a distinct non-*T. monococcum* phenotype and were ~ 60% larger.
- The heads possessed ~ 25% more spikelets.
- Leaves were ~ 25% smaller.
- Heading date was earlier than that of trisomics for other chromosomes, but head two weeks later than that of diploids.
- Possessed ~ 20% fewer tillers.
- Highly sterile (~96%).
- The head phenotype permits easy identification and distinction from diploids and other trisomics.

TRISOMICS FOR CHROMOSOME 4A

- Normal growth habit with ~ 40% reduction in size.
- On the average, heads were a little larger.
- The number of spikelets/head was the same.
- Leaves were about the same size or slightly larger.
- Like trisomics for chromosome 3A, heading date was ~ two weeks later.
- Tillering was reduced by ~ 40%.
- Fertility was ~ 7%.
- Except for breeding behaviour and fertility, they were more normal in phenotype than the other trisomics and were distinguishable from diploids but not from trisomics for the other chromosomes in triplicate.

TRISOMICS FOR CHROMOSOME 5A

- Possessed a normal growth habit with ~ 35% reduction in height.
- Heads were ~ 35% shorter.
- There were ~25% fewer spikelets/head.
- Heading date was three weeks later.
- Average fertility was 16%.

-The morphological features of these trisomics were very much like those of trisomics for chromosome 1A. They can be distinguished from diploids but not the other trisomics.

TRISOMICS FOR CHROMOSOME 6A

-Normal growth habit with an ~ 30% reduction in height.

-Heads were 25% smaller.

-There were ~ 20% fewer spikelets/head.

-Leaves were 25% shorter.

-Heading date was about three and a half weeks later.

-~ 35% reduction in tillering.

-Fertility was reduced by ~ 60%.

-These charateristics permitted easy distinction from diploids but not from other trisomics.

TRISOMICS FOR CHROMOSOME 7A

-The shortest of all trisomics; height was reduced by ~ 40%.

-Heads were as short as those in plants trisomic for chromosome 1A; head size was reduced by ~ 40%.

-The number of spikelets per head were also reduced by ~ 30%.

-The leaves were ~ 25% shorter than those in diploids. They were also much narrower than those in diploids and other trisomics (e.g. 40 - 60% narrower than in diploids). These traits were distinguishable during both the vegetative stage and in mature plants.

-Heading date was about four and a half weeks later than other trisomics.

-~ 40% fewer tillers.

-These trisomics were highly sterile (91.4%).

-The leaf and head phenotypes were distinct. These features, plus the very late heading permitted easy identification and distinction from all other trisomics.

Note: None of the trisomics; with the exception of trisomics for chromosome 7A, were distinguishable from the diploids until the late vegetative - fully grown stage.

B. Double and triple trisomics

The phenotypes of the double and triple trisomics deviated from those of the diploid progenitors to a greater extent than did those of the single trisomics. Compared with the single trisomics, double trisomics were less vigorous, smaller with fewer tillers and spikelets per head, had smaller leaves, headed later, and were completely sterile. The triple trisomics exhibited an even greater degree of deviation from normal in all these respects. These results are similar to the findings in *Datura* (Blakeslee 1934), tomato (Rick and Barton 1954), snapdragon (Sampson *et al.* 1961), barley (Tsuchiya 1960, 1983), rye (Kamanoi and Jenkins 1962), pearl millet (Gill *et al.* 1970) and most other diploid plant species.

VI. Breeding behaviour and fertility of trisomics of *T. monococcum*

A. Breeding behaviour

The breeding behaviour of the primary trisomics was studied by determining the frequency of trisomics among the progenies derived from self-fertilization of these aneuploids and reciprocal crosses between them and the diploid progenitors (Table 11). For all the trisomic lines, regardless of whether the primary trisomics were self-fertilized or crossed with diploids, the percent of progeny that were trisomic was much less than the expected 50%. With the exception of *G. max* (Palmer 1976) and *P. lagopus* (Sharma *et al.* 1985), these results were consistent with those for all other diploid plant species, including those that are highly tolerant of aneuploidy (Rick and Barton 1954; Janick *et al.* 1959; Tsuchiya 1960; Dhillon and Garber 1960; Kamanoi and Jenkins 1962; Koornneef and vander Veen 1983; Khush *et al.* 1984 Singh *et al.* 1984). Moreover, as in all other diploid plant species, except Birdsfoot treefoil (Chen and Grant 1968b), there was a significant difference in the frequency of trisomics among the progenies of reciprocal crosses. The results of the *2n*♀ x *2n+1*♂ crosses were the same for all trisomic lines (Table 11). With one exception, trisomic progeny were not produced in these crosses. In

Table 11. Number of diploid and trisomic progenies and percent of the latter from self-fertilization of primary trisomics of *T. monococcum* and reciprocal crosses between them and diploids.

Trisomic for Chromosome	Trisomic line*	Self-fertilization of trisomics**			Trisomic♀ x Diploid♂***			Diploid♀ x Trisomic♂****		
		No.$2n$	No.$2n+1$	% $2n+1$	No.$2n$	No.$2n+1$	% $2n+1$	No.$2n$	No.$2n+1$	% $2n+1$
1A	50	310	39	11.2	123	17	13.8	107	0	0
2A	3	233	44	15.9	154	25	14.0	119	0	0
3A	26	89	5	5.6	37	3	7.5	41	0	0
	40	72	4	5.5	61	5	7.6	37	0	0
4A	4	91	4	4.2	33	2	5.7	30	0	0
	6	108	4	3.4	41	2	4.7	43	0	0
5A	28	194	14	6.7	90	7	7.2	73	0	0
	51	151	15	9.0	89	12	11.4	94	0	0
6A	8	271	31	10.3	114	12	9.5	105	0	0
	16	219	29	11.3	119	14	10.5	125	1	0.8
	29	153	24	13.6	97	11	10.2	97	0	0
	33	199	26	11.2	120	19	13.5	120	0	0
	36	156	26	14.3	129	24	15.7	118	0	0
	42	177	23	11.4	112	19	14.5	66	0	0
	57	196	25	11.3	97	17	14.5	111	0	0
7A	10	170	11	6.1	26	2	7.1	62	0	0
	22	211	19	7.5	79	8	9.2	91	0	0
1A or 5A	7	215	33	13.7	123	24	16.3	94	0	0
	20	284	23	7.5	100	6	5.7	94	0	0
	38	207	22	9.6	94	9	8.7	104	0	0
Aveage				9.5			10.4			0.04

* No data on trisomics in lines 34, 39, 47, 52 and 58 which were lost early in the study.

** The self-fertilization data are pooled results from four years beginning in 1986.

*** The numbers of diploids and trisomics are pooled values for 1986 and 1987.

**** The results of the cross represent the pooled values for 1986 and 1987.

this respect, these results are like those obtained in barley (Tsuchiya 1960), spinach (Janick *et al.* 1959), ryegrass (Meijer and Ahloowalia 1981) and millet (Singh *et al.* 1984). The frequency of trisomics was much higher in trisomic♀ x diploid♂ crosses and varied from one trisomic line to another. It ranged from 4.7% trisomics among the progeny of plants trisomic for chromosome 4A to 16.3% among the progeny produced by those trisomic for either chromosome 1A or 5A. These results were different from those in all other diploid species in that both the range of frequencies of trisomics in different trisomic lines and the average frequency (10.4%) of these aneuploids was much lower. For example, in *H. spontaneum* (Tsuchiya 1960) and *S. cereale* (Kamanoi and Jenkins 1962), the of frequencies of trisomics ranged from 9.8 to 27.2 and 16.0 to 39.3 with average frequencies of 22.7 and 26.5, respectively. A comparison of the progenies produced by self-fertilizing *T. monococcum* trisomics and those derived from $2n+1$♀ x $2n$♂ crosses (Table 11) revealed that trisomics occurred with almost the same frequencies in both cases. This indicates that the source of the male gametes is not the determining factor in the breeding behaviour of primary trisomics in *T. monococcum*.

T. monococcum trisomics produced n and $n+1$ meiotic products in approximately equal frequencies during microsporogenesis (Table 8). It is assumed that the meiotic chromosome behaviour is the same during megasporogenesis. Therefore post-meiotic factors must be responsible for the reduction (<50%) in the frequency of trisomic progeny. That trisomic progeny were not produced in $2n$♀ x $2n+1$♂ crosses indicated that $n+1$ male gametophytes are either not produced or they abort. On the other hand they either fail to germinate, grow slower or mature later than n pollen grains and thus cannot effect fertilization. The exact cause or causes of lack of functional $n+1$ male gametes was not investigated. Almost all the seeds produced from these crosses were normal in size with approximately 100%

germination, indicating that post-zygotic factors were not responsible for the absence of trisomic progeny. The low frequency of trisomics among progenies of self-fertilized trisomics and those derived from $2n+1$♀ x $2n$♂ crosses was, in part, attributed to failure of some of the small trisomic seeds to germinate. In all the seven trisomic lines, approximately 40% of the smaller seeds and 10% of the larger seeds failed to germinate. With few exceptions, the latter seeds produced diploid progeny. Reduced viability of $n+1$ female meiotic products and gametophytes, and reduced viability or lethality of some $2n+1$ zygotes and embryos may also have contributed to the paucity of trisomic progeny in these crosses. Lethality and low vigor of trisomic seedlings may have caused some of the trisomic seedlings to die shortly after germination.

The breeding behaviour of the different trisomics was unrelated to the size of the chromosome in triplicate. Although chromosome 2A is the longest chromosome in the genome, trisomy for this chromosome showed the highest frequency of trisomics among their self-fertilized and trisomic♀ x diploid♂ progenies. Chromosomes 4, 5, and 6 are almost the same size (Table 5); however in each case, trisomy for these chromosomes showed variable frequencies of trisomic progeny. Average frequencies of trisomics among their self-fertilized progenies were 3.8, 7.9, and 11.9%, respectively for these chromosomes. Similar results were observed in the progenies of trisomic♀ x diploid♂ crosses. These results were found to be consistent with those reported by Vari and Bhowal (1986) in *P. americanum*.

A study of the breeding behaviour of trisomics is necessary if one is to maintain these aneuploids and understand the extent to which they can be used in cytogenetic analyses. All *T. monococcum* trisomics produced trisomic progeny with a very low to low frequency. For example, trisomics for chromosome 7A produced trisomic progeny with a frequency of approximately 7%. In order to obtain 7 of these aneuploids for maintenance of the trisomic line and utilization in crosses to appropriate diploids for purposes of cytogenetic analyses, about 100 plants have to be grown. Each of these plants must be analyzed cytologically to select the trisomics. The same is true for each of the other six trisomic lines.

B. Fertility

Fertility levels in the trisomics were determined by calculating seed set per floret on self-fertilized plants in 1986 and 1987 (Table 12). The data for the two years were not significantly different within the various lines. Consistent with the observations in other diploid plant species (Khush 1973; Singh *et al.* 1984; Sharma *et al.* 1985), all primary trisomics in *T. monococcum* were significantly less fertile than diploids. The seed fertility of the *T. monococcum* trisomics was much lower than the ~65 to 90% seed fertility in barley trisomics (Tsuchiya 1960). The data in Table 12 reveals that the percent fertility ranges from 3.8% for trisomics for chromosome 1A to 40.6% for trisomics for chromosome 2A. The differences in the fertility levels between the lines could be due to genetic factors. Differences in fertility levels were observed among lines that were trisomic for the same chromosome. For example, lines 42 and 8 which are trisomic for chromosome 6A showed 29.4% and 41.7% fertility, respectively; lines 10 and 22 which are trisomic for chromosome 7A had fertility levels of 5.3% and 11.8%, respectively. These differences may be due to the heterozygous genetic background of the different trisomic lines. The low levels of fertility of lines trisomic for chromosomes 1A, 3A, 4A, and 7A made their maintenance and use in cytogenetic analyses difficult. In fact, this, together with a low frequency of trisomic progeny, resulted in the loss of all the trisomic lines in which chromosome 3A or 4A was present in triplicate. The low fertility and breeding behaviour of the other five trisomics made their

Table 12. Fertility level as measured by percent seed set on self-fertilized trisomic plants.

Trisomic for chromosome	Trisomic line*	1986			1987			Ave.
		No. heads	No.seeds per floret	% fertility	No. heads	No.seeds per floret	% fertility	fertility
1A	50	37	22/610	4.0	14	8/232	3.5	3.8
2A	3	8	97/240	40.0	13	282/520	41.1	40.6
3A	26	5	13/224	5.8	6	11/240	4.7	5.3
	40	9	26/432	5.6	-	-	-	5.6
4A	4	7	22/262	7.8	6	16/232	6.4	7.1
	6	8	22/271	7.5	3	6/120	4.7	6.1
5A	28	11	42/213	16.4	14	51/233	17.9	17.2
	51	20	50/340	14.7	26	60/440	13.6	14.2
6A	8	9	150/354	42.4	18	356/876	44.0	41.7
	16	9	132/372	35.5	18	215/708	30.0	32.8
	29	7	92/215	30.0	9	110/242	31.3	30.3
	33	11	120/388	30.9	10	140/340	41.2	36.1
	36	12	161/396	40.7	21	275/800	34.0	37.4
	42	10	89/230	28.2	7	74/168	30.6	29.4
	57	9	84/360	24.4	10	140/400	35.0	29.7
7A	10	7	15/208	7.2	8	8/240	3.3	5.3
	22	6	39/268	14.6	9	32/362	9.0	11.8
1A or 5A	7	26	90/968	9.3	16	88/656	13.0	11.2
	20	9	96/452	21.0	9	46/400	11.5	16.3
	38	7	22/244	9.0	10	79/402	20.0	14.5
Diploid		10	249/250	99.6	12	299/300	99.6	99.6

* No data on trisomics in lines 34, 39, 47, 52, and 58 which were lost early in the study.

maintenance and use in cytogenetic analyses very time consuming and labor intensive.

VII. Unsuccessful attempts in identifying trisomics

A. Crosses between *T. monococcum* trisomic and *T. aestivum* ditelosomic lines

Hybrids derived from *T. aestivum*♀ x *T. monococcum*♂ crosses are viable, less vigorous, and regularly form 7II + 14I (Melburn and Thomson 1927; Kuspira, unpublished data). All 42 ditelosomic lines, one for each arm of each of the 21 chromosomes, have been established in *T. aestivum* var. Chinese Spring (Morris and Sears 1967). The kinds of chromosome associations formed during D - MI in hybrids derived from crosses between *T. monococcum* primary trisomics and these *T. aestivum* var. Chinese Spring ditelosomic lines should therefore permit unequivocal identification of the chromosome present in triplicate in a given *T. monococcum* trisomic line. If the chromosome present in triplicate is homologous with the telocentric chromosome in a particular ditelocentric line, a heteromorphic III + 6II + 14I should be observed in some of the meiocytes; 7II + 15I (1 telocentric and 14 normal chromosomes) should be seen at D - MI in the remaining cells in this hybrid. If the chromosome in triplicate is not homologous with the telocentric chromosome in a ditelocentric line, a homomorphic III + 1 heteromorphic II + 5 homomorphic IIs + 14Is should be seen in some meiocytes; a heteromorphic II + 6 homomorphic IIs + 15Is should be observed in the remainder of the meiocytes in this hybrid. Interspecific crosses are usually successful only when the polyploid species is used as the female parent because endosperm development is more or less normal. The reciprocal crosses are rarely successful because of the failure of or reduced endosperm formation (Cooper and Brink 1945; Cooper 1951; Esen and Soost 1973; Kuspira *et al.* 1985).

The failure of *T. monococcum* trisomics to form *n+1* male gametes or the inability of these gametes to effect fertilization precluded making crosses in the desired direction. Two hundred and thirty eight crosses were made involving each of the seven *T. monococcum* trisomic lines as the female parent and the 14 ditelosomic lines for the A genome chromosomes in *T. aestivum* as the pollen source. Approximately half (~360) of the minute embryos from each cross were rescued and cultured without success. The other half (~370) matured into small, paper-thin seeds that did not germinate. This approach to identifying trisomics was therefore abandoned.

B. Phenotypes of biochemical markers

Proteins and enzymes, including isozymes, are coded by structural genes. In *T. aestivum*, the mode of inheritance of many of these biochemical markers as well as their chromosomal locations have been determined (Hart 1982, 1988; McIntosh 1988a and b). The isozymes, MDH, GPI-1, SOD-1, GOT-2, GOT-3, PGM-1, ACO-2, and EP, specified by genes on chromosomes of the A genome in *T. aestivum*, were used in an attempt to identify the chromosomes in triplicate in the *T. monococcum* trisomics. The starch gel electrophoretic phenotypes of each of these enzymes was determined in lines 68 and 89 as well as in the diploids and trisomics from some of the trisomic lines. The phenotypic expressions of the 8 isozymes were easily detected in all the plants that were analyzed. Determination of whether a gene coding for a particular enzyme is on the chromosome in triplicate in a given trisomic, however, is dependent on quantitative differences in phenotypic expression between diploids and trisomics. Regardless of the isozyme, such quantitative differences either did not occur or were not discernable in any of the lines studied. This approach, therefore, did not permit the identification of the chromosome present in triplicate in the trisomics examined. Alternate electrophoretic methods e.g., polyacrylamide gel electrophoresis, will have to be used to identify these aneuploids using biochemical markers.

C. N-banding

N-banding of *T. monococcum* chromosomes was unsuccessful. B.S. Gill (personal communication) and others had the same experience.

N-banding in *T.aestivum* shows that many of the N-bands occupy the same positions as C-bands (Gerlach 1977; Endo and Gill 1984a). These studies implied that at least two classes of constitutive heterochromatin occur in wheat. Endo and Gill (1984a) and Schlegel and Gill (1984) confirmed this implication and showed that N-bands possess $(GAA)_n(GAG)_n$ satellite sequences. Since no one has been successful in N-banding chromosomes of *T. monococcum*, this indicates that the N-band satellite sequences either do not exist in this diploid species or are present in such small amounts at the N- and C-banding regions that preclude their detection. Regardless which of these alternatives is true, the predominant satellite sequence in *T. monococcum* chromosomes is that which stains with C-banding protocols and yet to be idetified.

CONCLUDING REMARKS

Primary trisomics and telocentric trisomics have been very useful in cytogenetic analyses and used extensively in such studies in *L. esculentum* (Lesley 1932; Rick *et al.* 1964; Young *et al.* 1987), *H. spontaneum* (Tsuchiya 1960, 1983; Kaiser and Friedt 1989), *O. sativa* (Khush *et al.* 1984; Ranjhan *et al.* 1988), and many other diploid plant species (Khush 1973). Such studies have been nonexistent in *T. monococcum*. A complete series ($n=x=7$) of primary trisomics in *T. monococcum* was generated in a conventional manner so as to increase our knowledge of the genetics of the genome of *T. monococcum* and to cytogentically analyze its genome, which is purported to be the A genome in polyploid wheats.

Neither the phenotypic studies nor the analyses of standard karyotypes conducted in this investigation permitted an unequivocal identification of the chromosomes of *T. monococcum* and its trisomics. Of the available banding techniques, only the fluorescent, Giemsa C- and N-banding ones have been widely used in plants (Vosa 1974; Gerlach 1977; Greilhuber 1977). C-banding, which stains constitutive heterochromatin (Pardue and Gall 1970; Hsu 1973), has been the method of choice because it produces the highest resolution in most plant species, including those in the genus *Triticum* (Gill 1987; Simeone *et al.* 1988). Application of C-banding and the silver staining method of Lacadena *et al.* (1984) led to the following conclusions: (i) Trisomics for chromosome 3A were not found among the trisomic lines studied cytologically. This may either have have been the result of chance or to the fact that these aneuploids were lost before they could be identified. (ii) Of those trisomic for each of the six remaining chromosomes, four were positively identified. They were those trisomic for chromosome 2A, 4A, 6A and 7A. (iii) Trisomics for the SAT chromosomes 1A and 5A could not be unequivocally distinguished because of C-banding polymorphisms. *In situ* hybridization using a specific rDNA probe permitted a more specific identification of the SAT chromosomes in three of six lines believed to be trisomic for chromosome 1A or 5A. Line 50 is most likely trisomic for chromosome 1A while lines 28 and 51 are, in all likelihood, trisomic for chromosome 5A. Additional molecular and/or cytogenetic information is necessary to unequivocally identify the specific SAT chromosome present in triplicate in the remaining three lines. Since C-band polymorphism exists in different true-breeding lines, the establishment of a complete series of trisomics in true-breeding line 89, which is currently underway, should permit the unequivocal identification of trisomics for chromosomes 1A or 5A. Cytogenetic studies using genes known to be located on chromosome 1A or 5A in *T. aestivum* and therefore on their homologous counterparts in *T. monococcum*, should also permit determination of whether a line trisomic for the SAT chromosomes carries chromosome 1A or 5A in triplicate. For example, in *T. aestivum*, the only chromosome in the A genome that carries a major gene (*Vrn 1*) for growth habit is 5A (Unrau 1950; Law *et al.* 1976; Maystrenko 1980). In *T. monococcum*, growth habit is determined by one major gene (Kuspira *et al.* 1986a). Therefore, it is likely that the major gene for growth habit in *T. monococcum* is *Vrn 1* and is located on chromosome 5A. Analysis of F_2 and/or testcross data from crosses between the spring growth-habit lines trisomic for chromosome 1A or 5A and true-breeding winter growth-habit diploid lines should facilitate identification of the SAT chromosome in triplicate in a given trisomic line.

It should be noted that the genomes of *T. monococcum* and *T. urartu* are very similar (Chapman *et al.* 1976; Dvořák 1976, 1988; Kerby and Kuspira 1988b). Therefore, it is possible that *T. urartu*, and not *T. monococcum*, is the source of the A genome in the polyploid wheats. A comparison of Ag-NOR staining, *in situ* hybridization using rDNA probes, and C-banding patterns of these two diploid species and those of *T. turgidum* and *T. aestivum* may help resolve the current impasse.

The establishment of this nearly complete series of primary trisomics bodes well for cytogenetic analysis of *T. monococcum*. It is fervently hoped that these trisomic lines, upon propagation and availability, will usher in an era in wheat cytogenetics that will facilitate a thorough analysis of the A genome.

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